CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being hand delivered
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Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on ___2/13/04___

BY: _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Schultz et al.	Confirmation No.	2759
Serial No.:	10/074,745	Group Art Unit:	1639
Filed:	2/11/2002		
For:	Combinatorial Synthesis and Screening of Non-Biological Polymers	Examiner:	M. Garcia Baker

Santa Clara, California
February 12, 2004

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ADAM L. SAFIR

UNDER 37 C.F.R. §1.132

I, Adam L. Safir, hereby declare as follows:

1. I am the Polymer Synthesis Group Leader at Symyx Technologies, Inc., 3100 Central
    Expressway, Santa Clara, CA 95051. I own Symyx common stock and receive stock
    options as part of my compensation at Symyx. I make this declaration in support of
    Applicants' response to the issues raised by the Office.

2. I graduated from the University of California at Berkeley with a Ph.D. in Synthetic Chemistry in 1996 and from the University of California at Santa Barbara with a Bachelor of Science in Chemistry in 1991. I worked as a Visiting Scholar at the Polymer Science Department at the University of Massachusetts at Amherst from 1994-1996. I have authored or co-authored over 20 publications, many of which are related to polymerization. Examples include: U.S. Patent 6,566,461, to Freitag et al., issued May 20, 2003, and entitled "Methods for Parallel Semi-Continuous or Continuous Reactions"; U.S. Patent 6,559,255, to Klaerner et al., issued May 6, 2003, and entitled "Controlled Free Radical Emulsion and Water-Based Polymerizations and Seeded Methodologies Controlled, Stable Free Radical Emulsion Polymerization Processes"; U.S. Patent 6,485,692, to Freitag et al., issued November 26, 2002, and entitled "Continuous Feed Parallel Reactor"; and U.S. Patent 6,475,391, to Safir et al., issued November 5, 2002, and entitled "Rapid Characterization of Polymers for Combinatorial Library Synthesis". My CV is attached hereto as Exhibit A. I joined Symyx in 1997.

3. I have reviewed the April 9, 2003 Office action, the October 9, 2003 Amendment C, the December 4, 2003 Supplemental Amendment D, the currently pending claims and the specification in the above-identified patent application. I have also reviewed the various attachments to this Declaration.

4. I understand that the claims in this patent application (*e.g.,* independent claims 94, 121, 124, 127, 135 and 163, together with claims depending therefrom) are directed to methods for preparing and evaluating arrays of polymeric materials. As I read the claims, each of the methods requires at least preparing an array comprising first and second non-biological organic polymers on a substrate, by delivering a component of one non-biological organic polymer to a first region of a substrate, delivering a component of a second non-biological organic polymer to a second region on the substrate, and polymerizing to form the first non-biological organic polymer at the first region of the substrate, and the second non-biological organic polymer at the second region of the substrate.

4a. Additionally, claim 94 requires that the first and second non-biological organic polymers are copolymers or higher-order polymers, with the first non-biological organic copolymer being different from the second non-biological organic copolymer. The method of claim 94 requires that the first non-biological copolymer comprises a first component and a second component in the first region of the substrate, and that the second non-biological copolymer comprises a first component and a second component in the second region of the substrate and that the first and second polymerization reactions occur simultaneously.

4b. Claim 121 also requires that the polymers are copolymers or higher-order polymers and that the polymerization reactions occur simultaneously, with the further limitation that the monomers are delivered to ten or more regions of the substrate and form ten or more different non-biological organic copolymers on the substrate, and screening the ten or more non-biological organic copolymers for a property of interest selected from the group consisting of a thermal property, a mechanical property, a morphological property, a chemical property, an optical property, a magnetic property and an electrical property.

4c. Claim 124 also requires that the polymerization reactions occur simultaneously and that the polymers are copolymers or higher-order polymers different from each other with the additional limitation that the delivered monomers of the first and second non-biological organic copolymers react without linear, stepwise coupling thereof, to form the first and second non-biological organic copolymers.

4d. Claim 127 requires that the polymerization reaction conditions at the first region and the second region of the substrate are independently controlled.

4e. Claim 135 also requires that the polymers are copolymers or higher-order polymers, with the further limitation that the monomers are delivered to ten or more regions of the substrate and form ten or more different non-biological organic copolymers on the substrate, and that the first component of the ten or more non-biological organic copolymers is delivered in a gradient of stoichiometries to ten or more regions of the substrate.

4f. Claim 163 is specifically directed to polycarbonate copolymers, and requires that the ten or more polymers are polycarbonate polymers being different from each other. The array is prepared by simultaneously delivering a first component and a second component of a first polycarbonate polymer to a first region of the substrate, simultaneously delivering a first component and a second component of a second polycarbonate polymer to a second region of the substrate, simultaneously delivering a first component and a second component of a third polycarbonate polymer to a third region of the substrate, simultaneously delivering a first component and a second component of a fourth polycarbonate polymer to a fourth region of the substrate, simultaneously delivering a first component and a second component of a fifth polycarbonate polymer to a fifth region of the substrate, simultaneously delivering a first component and a second component of a sixth polycarbonate polymer to a sixth region of the substrate, simultaneously delivering a first component and a second component of a seventh polycarbonate polymer to a seventh region of the substrate, simultaneously delivering a first component and a second component of an eight polycarbonate polymer to an eighth region of the substrate, simultaneously delivering a first component and a second component of a ninth polycarbonate polymer to a ninth region of the substrate, simultaneously delivering a first component and a second component of a tenth polycarbonate polymer to a tenth region of the substrate, simultaneously polymerizing the delivered first and second components of each of the ten or more polycarbonate polymers to form the ten or more polycarbonate polymers at the ten or more regions of the substrate,

respectively, controlling the polymerization reaction conditions independently for each of the ten or more regions, and screening the polymers in parallel for a chemical property.

5. In my opinion, the state of the art for polymerization chemistry is well developed, as exemplified by the references attached as Exhibits B-F:

    Exhibit B - United States Patent No. 6,584,832 (2003);

    Exhibit C - "Separation Approaches Toward Rapid and Complex Molecular Characterization of Diverse Polymers," (2003);

    Exhibit D - PCT Patent Application WO 02/14377 (2002);

    Exhibit E - United States Patent Application 10/333,065, Publication No. US 2003/0157566 A1 (2003); and

    Exhibit F - "Water-Compatible Molecularly Imprinted Polymers Obtained via High Throughput Synthesis and Experimental Design" (2003).

6. As I read the reference, Exhibit B (of which I am a co-author) discloses the thermally initiated emulsion polymerization of a 96-member library of polymers. Table 14 and Column 84, lines 5-62 shows the library design and synthesis steps, in which the components of the 96 different polymerization mixtures were dispensed and polymerized - 24 different styrene polymers, 24 different butyl acrylate polymers, 24 different methyl methacrylate polymers and 24 different vinyl acetate polymers. The monomers were dispensed in a 96-well glass lined aluminum batch reactor in various ratios with other solution components, such as initiator, surfactant and/or water, which were then sealed and polymerized at 80°C for 4 hours.

7. In my opinion, the polymer library of Exhibit B was created using the inventions defined by the claims of the above-referenced patent application and in accordance with the methods described in the specification of the above referenced application. In my opinion, the library of polymers prepared in Exhibit B, contained at least two polymers that are different from each other (all created with different monomer amounts and monomer/other component ratios), contained ten or more polymers, the

mixtures were polymerized without stepwise coupling, the polymerization reactions occurred simultaneously and the polymerization reaction conditions at the first region and the second region of the substrate (a first and second well of the glass lined aluminum batch reactor) were independently controlled (by varying the monomer/other component ratios for each member of the library).

8. Based on my personal knowledge as a co-author, it is my opinion that no undue experimentation was necessary for carrying out the polymerization reactions described in Exhibit B.

9. As I read the reference, Exhibit C (whose authors are colleagues of mine at Symyx Technologies, Inc.) discloses the thermally initiated solution polymerization of a 96-member library of 2-hydroxyethyl methacrylate and styrene polymers (8 of the members were homopolymers of styrene, 8 of the members were homopolymers of 2-hydroxyethyl methacrylate, and 80 of the members were copolymers of styrene and 2-hydroxyethyl methacrylate). Figure 7.1 shows the library design, in which the ratios of the two monomers to each other as well as to the initiator were varied. The solutions were dispensed in a 96-well glass lined aluminum batch reactor, which was sealed and polymerized at 70°C for 12 hours.

10. In my opinion, the polymer library of Exhibit C was created using the inventions defined by the claims of the above-referenced patent application and in accordance with the methods described in the specification of the above referenced application. In my opinion, the library of polymers prepared in Exhibit C, contained ten or more copolymers that are different from each other (all created with different monomer amounts and monomer/initiator ratios), the mixtures were polymerized without stepwise coupling, the polymerization reaction conditions at the first region and the second region of the substrate (a first and second well of the glass lined aluminum batch reactor) were independently controlled (by varying the monomer/initiator ratios for each member of the library), and the first component of the ten or more non-

biological organic polymers was delivered in a gradient of stoichiometries to ten or more regions of the substrate (for example, 12 different amounts of styrene, ranging from 0 to 100% of the monomer compositions).

11. Based on my review of this reference alone, and based on my personal knowledge as a colleague of the authors, it is my opinion that no undue experimentation was necessary for carrying out the polymerization reactions described in Exhibit C.

12. As I read the reference, Exhibit D (whose authors also include colleagues of mine from Symyx Technologies, Inc.) is directed to a method of making an array of nanoparticular dispersion formulations, which includes making arrays of co-polymers through simultaneous polymerization. The Exhibit describes two 96-member libraries. The first library is shown in the Table entitled "Library 1: composition (S/AA/DMAEM [mol% of feed]" on pages 37 and 38. The polymers of the library were comprised of styrene, acrylic acid and dimethylaminoethylmethacrylate. Seven members of this library were copolymers of styrene and acrylic acid, eight members of the library were copolymers of styrene and dimethylaminoethylmethacrylate and ten members of the library were copolymers of acrylic acid and dimethylaminoethylmethacrylate (25 total members were copolymers, 3 members were homopolymers and the remainder were higher order polymers). The second library is shown in the Table entitled "Library 2: composition (S/AA/4-VP [mol% of feed]" on pages 39 and 40. The polymers of the library were comprised of styrene, acrylic acid and 4-vinyl pyridine. Seven members of this library were copolymers of styrene and acrylic acid, eight members of the library were copolymers of styrene and 4-vinyl pyridine and ten members of the library were copolymers of acrylic acid and 4-vinyl pyridine (25 total members were copolymers, 3 were homopolymers and the remainder were higher-order polymers). As discussed on page 41, the libraries were prepared using a reaction block containing 96 vials. The vials were loaded robotically with monomer, solvent and initiator, sealed, heated to a temperature of about 70 °C and allowed to react for about 6 hours.

13. In my opinion, the polymer library of Exhibit D was created using the inventions defined by the claims of the above-referenced patent application and in accordance with the methods described in the specification of the above referenced application. In my opinion, the library of polymers prepared in Exhibit D were prepared on a substrate (reaction block), by delivering a first component and a second component of one non-biological organic polymer (for example, styrene and acrylic acid) to a first region of a substrate (vial in the reactor block), delivering a first component and a second component of a second non-biological organic polymer (for example, dimethylaminoethylmethacrylate and acrylic acid) to a second region on the substrate (vial in the reaction block), and simultaneously polymerizing the components of the first and second polymers. Furthermore, at least two of the copolymers were different from each other, there were ten or more copolymers (93 polymers comprising 2 or more monomers in each library), the polymerizations occurred without stepwise coupling and the first component of the ten or more non-biological organic polymers was delivered in a gradient of stoichiometries to ten or more regions of the substrate (for example, the libraries were designed to cover 96 different combinations of the three monomers of the design, ranging in composition amounts from 0 to 100% for each monomer in the system).

14. Based on my review of the reference alone, and based on my personal knowledge as a colleague of some of the authors, it is my opinion that no undue experimentation was necessary for carrying out the polymerization reactions described in Exhibit D.

15. As I read the reference, Exhibit E is directed to a combinatorial process for preparing an array of aqueous polymer dispersions by emulsion polymerization. The Exhibit describes the creation of a 16-member library polymerized in a reaction block. Example 3, found at paragraph 113 on page 8, describes the semi-continuous polymerization of 16 formulations. The monomer compositions, as shown in Table 1 on page 7, are 2-ethylhexyl acrylate and acrylic acid in 4 different ratios, n-butyl acrylate and acrylic acid in 4 different ratios, n-butyl acrylate, acrylic acid and styrene in 4 different ratios, and n-butyl acrylate, acrylic acid and methyl methacrylate in 4

different ratios. The example utilized a reactor block with 16 reactors, in which the monomers, initiator and emulsifier were dispensed and emulsified in water. One quarter of each mixture was then dispensed into a second rector block having 16 reactors. Polymerization was initiated thermally at 90 °C, with the remainder of the mixtures being added by pipette over 120 minutes.

16. In my opinion, the polymer library of Exhibit E was created using the inventions defined by the claims of the above-referenced patent application and in accordance with the methods described in the specification of the above referenced application. In my opinion, the library of polymers prepared in Exhibit E were prepared on a substrate (reaction block), by delivering a first component and a second component of one non-biological organic polymer (for example, n-butyl acrylate and acrylic acid) to a first region of a substrate (reactor vessel in the reactor block), delivering a first component and a second component of a second non-biological organic polymer (for example, 2-ethylhexyl acrylate and acrylic acid) to a second region on the substrate (reactor vessel in the reaction block), and simultaneously polymerizing the components of the first and second polymers. Furthermore, at least two of the copolymers were different from each other, there were ten or more copolymers (sixteen in Example 3), the polymerizations occurred without stepwise coupling and the first component of the ten or more non-biological organic polymers was delivered in a gradient of stoichiometries to ten or more regions of the substrate (for example, acrylic acid was delivered in an amount of 20, 40, 60 and 80 mg for each of 4 different monomer combinations).

17. Based on my reading of the Exhibit, it is my opinion that there is no discussion of the need for any undue experimentation necessary for carrying out the polymerization reactions described in Exhibit E.

18. As I read the reference, Exhibit F describes the high throughput synthesis of molecularly imprinted polymer sorbents. The Exhibit describes creating a library of 80 polymers by varying a composition of 2-hydroxyethyl methacrylate (HEMA),

methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA). Figure 1 shows the general procedure for the synthesis of the library, including dispensing the polymerization mixtures into a 96-well plate, and simultaneously polymerizing the library, either thermally or with UV light, while the library resides in or on the plate. Table 2 describes 20 of the library compositions. Five different polymer compositions were made, and one of four different porogens was combined with each set of five polymer compositions, creating 20 total compositions. An imprinted and a non-imprinted set for the twenty compositions was made, resulting in 40 total compositions, and each sample had a replica, resulting in 80 total members of the library. An initiator solution was first dispensed into a 96 well microtiter plate, followed by the addition of solutions of the functional monomers (HEMA and MAA), followed by the addition of EDMA cross-linker. The plate was then sealed and heated in an oven for 24 hours at 50 °C.
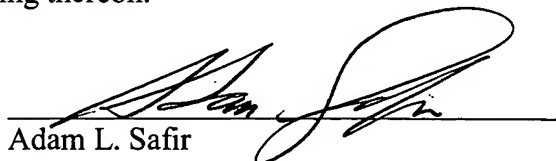
19. In my opinion, the polymer library of Exhibit F was created using the inventions defined by the claims of the above-referenced patent application and in accordance with the methods described in the specification of the above referenced application. In my opinion, the library of polymers prepared in Exhibit F were prepared on a substrate (reaction block as shown in Figure 1), by delivering a first component and a second component of one non-biological organic polymer (for example, EDMA and MAA) to a first region of a substrate (vial in the reactor block as shown in Figure 1), delivering a first component and a second component of a second non-biological organic polymer (for example, HEMA and MAA) to a second region on the substrate (vial in the reactor block as shown in figure 1), and simultaneously polymerizing the components of the first and second polymers. Furthermore, at least two of the copolymers were different from each other (the five different compositions contained different amounts of each, with some having no HEMA as shown in Table 2), there were ten or more copolymers (80 total polymers synthesized) and the polymerizations occurred without stepwise coupling.

20. Based on my reading of Exhibit F, it is my opinion that there is no discussion of the need for any undue experimentation necessary for carrying out the polymerization reactions described therein.

21. It is my opinion that one of ordinary skill in the art has worked as a scientist in a polymer chemistry laboratory or that that person is expected to have a Ph.D. in Chemistry. It is also my opinion that a person of ordinary skill in the art would have been able to practice the inventions defined by the claims without undue experimentation. As shown in the Exhibits discussed herein, the polymerizations were all carried out in a scope similar to that disclosed in the specification, using formats similar to that disclosed in the specification, without reporting the need to overcome any problems that would require further experimentation. I believe that many known polymerization techniques and chemistries can be used to practice this invention, as is demonstrated in the Exhibits discussed herein, as the state of the art for polymerization is well developed.

22. In my opinion, a person of ordinary skill in the art would have appreciated that the *nature of the invention* defined by the pending claims relates to a protocol involving a format for preparing and screening arrays of non-biological organic polymers – that is completely general to, and independent of, the particular types of polymerization chemistries (*e.g.*, polymer structures) and polymerization reactions. As I understand it, it is the inherent unpredictability in the art of polymer structures that makes the invention particularly useful – because it provides a protocol and format that offers significant advantages for investigating polymers and polymerization reactions.

23. In my opinion, the specification provides substantial guidance relating to the particular aspects and techniques that relate to the particular *nature of the invention* that Applicants are claiming. Specifically, an overview of general and specific approaches is provided (*See* page 12, line 1 through page 17, line 4 of the specification), together with specific details regarding various component-delivery approaches. Among others, gas-phase chemical processes and liquid-phase chemical

processes and deposition techniques are disclosed in significant detail. These techniques are suited to delivery of monomers, including delivery techniques for solution-phase monomers (*e.g.* with a dispenser), and are taught as being suitable for delivery of non-biological polymeric components. (*See*, for example, page 29, line 24 through page 36, line 12 of the specification.) In my opinion, the specification also describes various approaches for isolation of predefined regions (*See*, for example, page 17, line 6 through page 22, line 6 of the specification). Reaction of monomers and optionally other delivered components appears to be described with respect to non-biological polymeric materials. (*See*, for example, page 38, line 27-33 of the specification). Various reaction protocols that are effective in connection with bulk polymerization reactions, such as stirring and/or pressurizing and/or heating during the reaction, are likewise disclosed. (*See*, for example: page 36, lines 17-21 and page 37, lines 25-28 (heating); page 36, lines 25-30 (mixing); and page 37, lines 23-25 (pressurizing)). Intermittent reaction processing steps are likewise disclosed. (*See*, for example, page 37, line 29 through page 38, line 3). It is my opinion that the specification also discloses that the arrays of non-biological polymeric materials can be screened according to many specifically-known techniques for specifically-known properties of interest. (*See*, for example, page 39, line 6 through page 43, line 31 of the specification). Moreover, the preparation of an array of non-biological polymers is exemplified in Example B – and, in my opinion, demonstrates the invention defined by the presently-pending claims.

24. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patents issuing thereon.

Date: _February 12_, 2004

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Adam L. Safir

ADAM L. SAFIR

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Berkeley, CA 94703
510-486-1793

EDUCATION

June 1996
University of California at Berkeley
Ph.D. Synthetic Chemistry
Thesis: 1,2-Olefin Insertion Polymerizations Initiated by Neutral
Palladium(II): Precursors to Conjugated Polymers
Thesis Advisor: Dr. Bruce M. Novak
GPA: 3.94 of 4.00

June 1991
University of California at Santa Barbara
B.S. Chemistry, Phi Lambda Upsilon
GPA: 3.87 of 4.00

EXPERIENCE

1997 to present
Distinguished Scientist & Polymer Synthesis Group Leader
Symyx Technologies
Specialty Polymers Division
Research: Development and implementation of combinatorial techniques
for polymer synthesis and rapid polymer characterization, with a focus on
reactor and software design. Application of combinatorial techniques
toward the discovery of new polymeric materials in the fields of home and
personal care, adhesives, coatings and specialty additives. Synthetic work
focused in the areas of emulsion polymerizations and living free radical
polymerizations.
Current Director: Dr. Damian Hajduk

1994 to 1996
Visiting Scholar
Department of Polymer Science and Engineering
University of Massachusetts at Amherst
Continuation of graduate research in the design and synthesis of palladium
complexes for 1,2-olefin-insertion polymerizations.
Advisor: Professor Bruce M. Novak

1991 to 1994	**Graduate Research** Department of Chemistry University of California at Berkeley Research: Design and synthesis of neutral palladium(II) complexes as initiators for 1,2-insertion polymerizations, block copolymerizations and carbon monoxide alternating copolymerizations with bicyclic olefins. Advisor: Professor Bruce M. Novak
1988 to 1991	**Undergraduate Research** University of California at Santa Barbara Research: Organic and organometallic synthesis toward the development of complexes containing iridium-silicon or rhodium-silicon bonds. Advisor: Professor Richard J. Watts
Summer 1991	**Summer Internship** Solar Energy Research Institute, Golden, CO. Research: Procedural development for the pyrolysis of biomass and the subsequent isolation of 1,6-anhydro-beta-D-glucopyranose. Director: Dr. Joe Bozell
1991 to 1993	**Graduate Student Instructorship** University of California at Berkeley Responsibilities: Three semesters as a teaching instructor for General Chemistry and Advanced Physical Organic Chemistry. Responsibilities included lecturing, supervising field work and laboratory experiments, as well as preparing and grading reports and exams.

HONORS

- Outstanding Teaching Award (Advanced Physical Organic Chemistry)
- Willard L. McRary Prize in Chemistry (June 1991)
- American Institute of Chemists Award (June 1991)
- UCSB Foundation Student Recognition Award (December 1990)
- Award of Merit in General Chemistry (June 1988)
- UCSB L&S Honors Society
- Chancellor's Recognition of Excellence (September 1987)

FELLOWSHIPS

- DOED Fellowship (1991 to 1994)
- UCSB Undergraduate Fellowship in Chemistry (1987 to 1991)
- Regents' Scholarship (1987 to 1991)

SKILLS

- Synthesis: Polymer, emulsion, organometallic. Schlenk line/Dry box techniques. Combinatorial Polymer Science.
- Analysis: NMR, IR, AA, IC, and UV/*vis* spectroscopies.
- Polymer Characterization: GPC, GPC/light scattering, TGA and DSC, DLS.

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PATENTS & APPLICATIONS (partial list)

1) Petro, Carlson, Safir, Nielsen, Dales, Lee.; "High-Temperature Characterization of Polymers with HPLC System Having Multiple Mobile-phase Reservoirs"; **US 6584832**; July 2003.

2) Nielsen, Kuebler, Lee, Safir, Petro; "Characterization of Non-Biological Polymers Using Flow-Injection Analysis with Light-Scattering Detection"; **US 6577392**; June 2003.

3) Freitag, Hajduk, Nielsen, Safir, Tiede; "Methods for Parallel Semi-Continuous or Continuous Reactions"; **US 6566461**; May 2003.

4) Klaerner, Safir, Nielsen Jandeleit, Huefner, Li, Dales, Vanbeek; "Controlled Free Radical Emulsion and Water-Based Polymerizations and Seeded Methodologies Controlled, Stable Free Radical Emulsion Polymerization Processes"; **US 6559255**, May 2003.

5) Dales, VanBeek, Hajduk Nielsen, Mansky, Safir; "Parallel Reactor with Internal Sensing and Method of Using Same"; **US 6548026**, April 2003.

6) Hajduk, Nielsen, Safir, Matsiev, McFarland, Mansky; "Multi-Temperature Modular Reactor and Method of Using Same"; **US 6528026**, March 2003.

7) Turner, Van Erden, Dales, Safir, Nielsen; "System for Creating and Testing Novel Catalysts, Reactions and Polymers"; **US 6508984**; January 2003.

8) Safir, Petro, Nielsen, Carlson; "Targeted Separation Protocols for Rapid Characterization of Polymers"; **US 6491823**; December 2002.

9) Petro, Safir, Nielsen, Lee; "Automated Sampling Methods with Integral Serial Sample Preparation for Rapid Characterization of Polymers"; **US 6492184**; December 2002.

10) Freitag, Hajduk, Nielsen, Safir, Tiede; "Continuous Feed Parallel Reacto"r; **US 6482909**, November 2002.

11) Safir, Petro, Nielsen, Leethoma, Frechet, McFarland; "Rapid Characterization of Polymers for Combinatorial Library Synthesis"; **US 6468806**; October 2002.

12) Safir, Petro, Nielsen; "Parallel Liquid Chromatography for Analyzing Combinatorial Libraries of Non-Biological Polymers"; **US 6461515**; October 2002.

13) Safir, Petro, Nielsen, Carlson; "Overlaid Injection for Rapid Characterization of Polymers"; **US 6454947**; September 2002.

14) Safir, Petro, Nielsen, Lee, Frechet; "Rapid Characterization of Polymers"; **US 6406632**; June 2002.

15) Lacy, McFarland, Safir, Turner, Van Erden, Wang; "Graphic Design of Combinatorial Material Libraries"; **EP 1080435**; February 2002.

16) Petro, Safir, Nielsen, Carlson, Schultz, Xiang; "High-Temperature Characterization of Polymers with HPLC System Having Multiple Mobile-phase Reservoirs"; **US 6350916**, February 2002.

17) Petro, Safir, Nielsen, Lee; "Automated Sampling Methods for Rapid Characterization of Polymers"; **US 6265226**; July 2001.

18) Petro, Safir, Nielsen, Dales, Carlson, Lee; "High-Temperature Characterization of Polymers"; **US 6260407**, July 2001.

19) Nielsen, Kuebler, Bennett, Safir, Petro; "Flow-Injection Analysis and Variable-Flow Light-Scattering Methods and Apparatus for Characterizing Polymers"; **US 6175409**; January 2001.

PUBLICATIONS

1) Safir, A. L.; Novak, B. M. "Living CO/Olefin Alternating Copolymerizations of Electron-Poor Bicyclic Olefins Initiated by Neutral Palladium(II) Alkyl Complexes: A Route to the Perfectly Alternating Copolymer of CO and Acetylene" *J. Am. Chem. Soc.*, **1998**, *120*, 643.

2) Safir, A. L.; Novak, B. M. "Living 1,2-Olefin-Insertion Polymerizations Initiated by Palladium(II) Alkyl Complexes: Block Copolymers and a Route to Polyacetylene-Hydrocarbon Diblocks" *Macromolecules,* **1995**, *28,* 5396.

3) Safir, A. L.; Novak, B. M. "Air- and Water-Stable 1,2-Vinyl-Insertion Polymerizations of Bicyclic Olefins: A Simple Precursor Route to Polyacetylene" *Macromolecules*, **1993**, *26*, 4072.

4) Djurovich, P. I.; Safir, A. L.; Keder, N. L.; Watts, R. J. "Synthesis and Structure of Fac-Tris[(8-Quinolyl)Dimethylsilyl]Rhodium(III) and Iridium(III)" *Inorganic Chemistry*, **1992**, *31*, 3195.

5) Djurovich, P. I.; Safir, A. L.; Keder, N. L.; Watts, R. J. "A New Class of Metal-Silicon Bonded Complexes - 8-(Dimethyl-Silyl)Quinoline Derivatives of Rhodium and Iridium" *Coordination Chemistry Reviews,* **1991**, *111*, 201.

(72) Inventors: SCHROF, Wolfgang; In den Schelmenäckern 38, 67271 Neuleiningen (DE). SEUFERT, Michael; Wellsring 32, 67098 Bad Dürkheim (DE). HEGER, Robert; Liegnitzerstrasse 3, 69124 Heidelberg (DE). IDEN, Rüdiger; Wichernstrasse 3, 67373 Dudenhofen (DE). HORN, Dieter; Schröderstrasse 69, 69120 Heidelberg (DE). KOLTZENBURG, Sebastian; Berliner Strasse 33, 67165 Waldsee (DE). NIELSEN, Ralph, B.; 5760 Harder Street, San Jose, CA 95129 (US). CARLSON, Eric; 303 College Avenue, Palo Alto, CA 94306 (US). CHANG, Han-Ting; Garnet Drive, Livermore, CA 94550 (US). PETRO, Miroslav; 1079 Prouty Way, San Jose, CA 95129 (US).

KUEBLER, Sigrid; 3100 Central Expressway, Santa Clara, CA 95051 (US).

(74) Agent: ISENBRUCK, Günter; Bardehle, Pagenberg, Geissler, Altenburg, Dost, Isenbruck, Theodor-Heuss-Anlage 12, 68165 Mannheim (DE).

(54) Title: PROCEDURE AND DEVICE TO DEVELOP NANODISPERSANTS

(57) Abstract: Disclosed is a method of making an array of n nanoparticular dispersion formulations, wherein said nanoparticular dispersion formulations each comprise -at least one nanodispersant, -at least one application media, -an active ingredient said method comprising the following steps: c) making said array of n nanoparticular dispersion formulations by c1) a parallelized solid solution route, or c2) a parallelized general precipitation route, or c3) a parallelized reactive precipitation route, d) parallelized, rapid serial or semi-parallel characterizing of said obtained n nanoparticular dispersion formulations; an array of at least 8 different nanoparticular dispersion formulations; a method of making an array of m nanodipersants by a parallel polymerization process; an array of at least 8 different nanodispersants; a method of making an array of n solid solutions by a parallelized solid solution route; and an array of at least 8 different.

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Procedure and device to develop nanodispersants

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Field of the invention

The present invention generally relates to an array of nanoparticular dispersion formulations and a method of making said array. The invention can be applied, for

15   example, to search new effective nanodispersants for a stabilization of poorly soluble or insoluble materials in application media, e.g., active ingredients as pharmaceuticals, crop protection agents, vitamins or dye stuffs. Once prepared, these nanoparticular dispersion formulations can be screened in parallel or in a rapid serial screening for stability, for example, by optical methods.

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Background of the invention

Nanoparticular systems are of interest because of very specific advantages, e.g., coloristic, rheological, nonlinear optical properties, bioavailability, etc.

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Dispersions of particles are generally obtained in two different ways.

Standard grinding processes starting from solid bulk materials do not typically result in particles with average diameters less than 0,5 μm. Particle size and

30   distribution depends on a variety of parameters like the type of mill or the crushing parts (e.g. silica) used. A further problem is to remove the crushing parts after milling. If smaller grinding fractions are needed, often the smaller crushing parts and grinding dust are left in the product yielding a heterogeneous system.

Because of the larger particle size of milled materials it is more difficult to find additives to stabilize a dispersion of these particles against agglomeration, flocculation, sedimentation and flotation.

5   An alternative is to start from the molecular solution and to form particles by precipitation. This process faces problems from Ostwald ripening (crystal growth) and/or particle agglomeration again resulting in sedimentation and/or flotation. Generally, the precipitation process is induced in a nucleation stage by changing the compatibility with the surrounding medium (solvent system), e.g., by chemical

10   reaction of the substrate, changing or mixing of solvents, changes in pH value, temperature, pressure, or concentration.

In order to stabilize particular systems, surface-active additives have to be used to inhibit crystal growth and agglomeration in particles of nanometer size. Typical

15   additives are low molecular weight tensides or oligomers yielding so-called solubilisates (micelles) with the drawback of very small content of substrate molecules. Solubilisates show no nucleation process at the beginning of particle formation but a micellar solution process of the substrate by the tenside molecules. Unfortunately, the solvation power of the tensides can induce

20   nucleation and crystal growth because of better transportation of substrate molecules through the solvent medium.

High molecular weight additives are e.g. protective colloids, amphiphilic copolymers, thickeners, etc. Whereas protective colloids stabilize particles against

25   agglomeration by coating the particle surfaces forming a repulsive interaction (steric and/or electrostatic) between particles and inhibit growth by blocking growing sites at the particle surface, thickeners stabilize kinetically by slowing down diffusion and particle collision rates.

30   In any case, these complex interactions in the colloidal state make it nearly impossible to predict an effective additive to stabilize a given substrate, neither from theoretical calculations nor from formulation experience.

An object of the present invention is therefore to provide an experimental strategy to solve the complex precipitation problem that is individual to particular types of substrates.

5   **Summary of the invention**

The present invention provides an array of n nanoparticular dispersion formulations and a method of making said array, wherein said nanoparticular dispersion formulations each comprise the following components

10

- at least one nanodispersant,
- at least one application media, and
- one poorly soluble or insoluble material in said application media or a combination of more than one different poorly soluble or insoluble materials
15   in said application media having a single defined ratio between said different poorly soluble or insoluble materials in a single array, typically referred to in the specification and claims as active ingredient;

said method comprising the following steps

20

c)   making said array of n nanoparticular dispersion formulations by

c1)   a parallelized solid solution route, or

c2)   a parallelized general precipitation route, or

c3)   a parallelized reactive precipitation route,

25   d) parallelized characterizing of said obtained n nanoparticular dispersion
          formulations,

wherein said active ingredient is the same in each of said n nanoparticular dispersion formulations; and

30   wherein $n \geq 2$.

In a preferred embodiment said method comprises the following steps

a) a method of making an array of m nanodispersants by a parallel
polymerization process of at least 2 monomers A and B,

b) characterizing said nanodispersants,

c) making said array of n nanoparticular dispersion formulations, and

d) parallelized characterizing of said obtained n nanoparticular dispersion
formulations.

In one embodiment of the present invention the array of n nanoparticular dispersion formulations is made by a parallelized solid solution route. In a second embodiment of the present invention said array of n nanoparticular dispersion formulations is made by a parallelized general precipitation route and in a third embodiment said array is made by a parallelized reactive precipitation route. Using the foregoing method an array of 2 or more different nanoparticular dispersion formulations on a substrate at known locations thereon is obtained, wherein said nanoparticular dispersion formulations comprise

- at least one nanodispersant,
- at least one application media, and
- an active ingredient.

The present invention uses, in some embodiments, an alloy of nanodispersants, which may be made by a method of making an array of m nanodispersants by a parallel polymerization process comprising the steps

a1) delivering at least 2 monomers A and B, and optionally other components appropriate for the employed polymerization process to each synthesis region on a substrate having k physically separate synthesis regions for m different nanodispersants,

a2)   simultaneously reacting said monomers and other useful components to
       form m different nanodispersants.

Using said method an array of 2 or more different nanodispersants on a substrate
at known locations thereon is obtained containing a (co)polymer of at least two
monomers A and B.

The present invention also provides an array of 2 or more nandispersants and a
method of making an array of m nanodispersants and an array of 2 or more solid
solutions and a method of making an array of n solid solutions.

Detailed description of the invention and preferred embodiment

The present invention provides an array of n nanoparticular dispersion
formulations and a method of making said array.

Generally, the array of n nanoparticular dispersion formulations, wherein said
nanoparticular dispersion formulations each comprise the following components

-   at least one nanodispersant,
-   at least one application media, and
-   an active ingredient,
wherein said active ingredient is the same in each of said n nanoparticular
dispersion formulations; and

is prepared by a method comprising the following steps

c)   making said array of n nanoparticular dispersion formulations by

       c1)   a parallelized solid solution route, or

       c2)   a parallelized general precipitation route, or

122

c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

wherein n is at least 2 and wherein at least one parameter selected from the group consisting of components employed, concentration of the components, temperature, reaction time, pH-value, other useful components and solvent, if employed, is different in each of said nanoparticular dispersion formulations.

In a preferred embodiment of the present invention the method of making an array of n nanodispersion formulations comprises the following steps

a)   a method of making an array of m nanodipersants by a parallel polymerization process of at least 2 monomers A and B,

c)   making said array of n nanoparticular dispersion formulations wherein at least one of said obtained nanodispersants is employed by

c1)   a parallelized solid solution route, or

c2)   a parallelized general precipitation route, or

c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

wherein m is at least 2.

In a more preferred embodiment said method comprises the following steps

a)   a method of making an array of m nanodipersants by a parallel polymerization process of at least 2 monomers A and B,

b)   characterizing said nanodipersants,

c)   making said array of n nanoparticular dispersion formulations wherein at least one of said obtained nanodispersants is employed by

    c1)   a parallelized solid solution route, or

    c2)   a parallelized general precipitation route, or

    c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

wherein either n and m are independently of each other at least 2.

The complex interactions between a nanodispersant, an active ingredient and an application media in the colloidal state make it impossible to predict an effective nanodispersant for a given active ingredient to stabilize, neither from theoretical calculations nor from formulation experience. The method of the present invention provides an experimental strategy to solve the individual complex colloidal stabilization problem of each formulation given in this disclosure. Applying said new method of the present invention the number of nanodispersants and dispersion formulations synthesized can be increased to more than 1000 a day, whereas the number of nanodispersants and dispersion formulations synthesized by conventional methods of the state of the art is about 2 per person. The probability to find an effective nanodispersant for a given active ingredient is therefore much higher by applying the method of the present invention. By said new method of the present invention new effective nanodispersants and new formulations can be discovered in a short development time.

Additionally, small amounts of the components employed (nanodispersant, active ingredient, etc.) are necessary to prepare said dispersion formulations by the method of the present invention. This is an important factor, especially if the components employed, for example the active ingredient, are expensive or available in limited quantities.

Moreover, the reaction conditions at different reaction regions can be varied in a controlled manner. As such, components employed, concentration of the components, temperature, reaction time, pH-value, other useful components and solvent, if employed, etc. can be varied from reaction region to reaction region on

5   the substrate. These advantages are important impacts for commercial success.

General aspects

The following terms which are used in the specification and techniques which are

10   employed in the parallelized reactions of the present invention have the following general meaning:

Substrate:

A material having a rigid or semi-rigid surface such as a parallel reactor. In many

15   embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different materials with, for example, dimples, wells, vials, raised regions, edged trenches, or the like. In some embodiments, the substrate itself contains wells, raised regions, edged trenches, etc., which form all or part of

20   the synthesis regions. Preferably, the synthesis regions on said substrate are wells on a microtiter plate reactor or vials of a parallel reactor, which is especially preferred, if a solvent is used in the process of the present invention.

Synthesis region:

25   The synthesis region is a predefined region which is a localized area on a substrate which is, was or is intended to be used for formation of a selected nanoparticular formulation. The synthesis region may have any convenient shape, e.g. linear, circular, rectangular, elliptical, cylindrical, wedge-shaped, etc.

30   *Array:*

Generally, the array of materials is prepared by successively delivering components of materials to predefined synthesis regions on a substrate, and substantially concurrently, reacting the components to form at least two materials.

In the context of this specification the meaning of "reacting" is any kind of interaction between different components for example due to ionic, steric or electrostatic properties of said components or a chemical reaction of individual components or between at least to of them. Said "materials" may be, for example,

5   formulations such as nanoparticular dispersion formulations, solid solutions etc. In one embodiment, for example, a first component of a first material is delivered to a first synthesis region on a substrate, and a first component of a second material is delivered to a second synthesis region on the same substrate. Each component can be delivered in either a uniform or gradient fashion to produce

10   either a single stoichiometry, or alternatively, a large number of stoichiometries within a single synthesis region. The process is repeated, with additional components, to form a vast array of components at predefined, i.e. known locations on the substrate. Thereafter, the components are concurrently reacted to form at least two materials. The components can be sequentially or

15   simultaneously delivered to the synthesis regions on the substrate using any of a number of different delivery techniques.

The array of of dispersion formulations and the array of solid solutions prepared

20   by parallel design and synthetic techniques are obtained on a substrate of the above mentioned general meaning. The embodiment of said substrate may be different depending on the objective.

Delivery systems

25   In the delivery systems of the present invention, a small, precisely metered amount of each reactant component is delivered into each reaction region. This may be accomplished using a variety of delivery techniques, either alone or in combination with a variety of masking techniques. Preferred delivery systems of the present invention are dispensers.

30

Delivery using a dispenser

Dispensers can be utilized to generate diverse combinations of reactant components in the form of droplets (liquids) or powder on a single substrate.

Commercially available micropipetting apparatus can be adapted to dispense droplet volumes of 5 nanoliters or smaller from a capillary. Such droplets can fit within a reaction region having a diameter of 300 μm or less when a non-wetting mask is employed. In some embodiments, the micropipette is accurately and

5   precisely positioned above the reaction region, as described below, before the reactant solution is deposited.

In a different embodiment, the present invention employs a solution depositing an apparatus that resembles devices commonly employed in the ink-jet printing field.

10   Such ink-jet dispensers include, for example, the pulse pressure type, the bubble-jet type and the slit-jet type. Such ink-jet dispensers which can be used in the present invention are described in WO 96/11878, which is incorporated herein by reference for all purposes.

15   In another embodiment, aqueous reactant solutions can for example be delivered from a reservoir of the substrate by an electrophoretic pump. In such a device, the thin capillary connects a reservoir of the reactant with a nozzle of the dispenser. A suitable electrophoretic pump is described in WO 96/11878.

20   Using the aforementioned dispenser systems, the reactants can be delivered to predefined regions on a substrate either sequentially or simultaneously. In a presently preferred embodiment, the reactants are simultaneously delivered to either a single predefined region on the substrate or, alternatively, to multiple predefined regions on the substrate.

25

Moving the dispenser with respect to the substrate

To deposit liquids consistently at precisely specified regions using a dispenser, a frame of reference common to the delivery instrument and the substrate is required. In other words, the reference coordinates of the instrument must be

30   accurately mapped onto the reference coordinates of the substrate. Ideally, only two reference points on the substrate are required to completely map the array of reaction regions. The dispenser instrument locates these reference points and then adjusts its internal reference coordinates to provide the necessary mapping. After

this the dispenser can move a particular distance in a particular direction and be positioned directly over a known region. Of course, the dispenser instrument must provide precisely repeatable movements. Further, the individual regions of the array must not move with respect to the reference marks on the substrate after the

5 reference marks have been formed. A detailed description of suitable moving techniques for moving the dispenser with respect to the substrate is given in WO 96/11878 which incorporated herein by reference for all purposes.

Further preferred embodiments of the delivery technique of the reactants and the

10 technique for moving the dispenser with respect to the substrate are described later in the specification.

Dispersion formulations

The present invention relates to a method of making an array of n nanoparticular

15 dispersion formulations, wherein said nanoparticular dispersion formulations each comprise the following components

- at least one nanodispersant,
- at least one application media, and

20 - an active ingredient;
wherein said active ingredient is the same in each of said n nanoparticular dispersion formulations.

Nanodispersant

25 The nanodispersant in the present invention is a compound which is compatible with both, the active ingredient and the application media. Such compounds are preferably water compatible. This means that substantially no macroscopic phase separation is observed, when the nanodispersant is mixed with the active ingredient and the application media. Such compounds are preferably selected

30 from the group consisting of oligomers and low and high molecular weight polymers with a polymerization degree of more than 2. Said nanodispersants are for example selected from the group consisting of protective colloids, amphiphilic copolymers, and thickeners. Said nanodispersants may be surface-active additives,

which are used to inhibit crystal growth and agglomeration in a particle size of nanometers. Whereas protective colloids stabilize particles against agglomeration by coating the particle surfaces forming a repulsive interaction (steric or/and electrostatic) between particles and inhibit growth by blocking growing sites at

5   the particle surface, thickeners stabilize kinetically by slowing down diffusion and particle collision rates. (Co)polymers which can act as nanodispersants are for example random copolymers of vinyl monomers, copolymers with controlled architecture/blocks, condensation polymers, etc.. Preferably said nanodispersants are prepared by polymerization of at least 2 monomers A and B, selected from the

10 group consisting of hydrophobic, neutral hydrophilic, cationic and anionic monomers. Preferably, one of said monomers is a hydrophilic monomer and a second monomer is a hydrophobic monomer.

Application media

15 The application media is the media wherein at least one active ingredient is formulated and employed in form of a nanoparticular system. Generally, the active ingredient is poorly soluble or insoluble in said application media. Homogeneous stable nanoparticular dispersion formulations are of interest because of very specific advantages, e.g. coloristic, rheological, bioavailability,

20 nonlinear optical properties, and other properties. The preferred application media of the present invention is an aqueous system, preferably, the aqueous system is pure water or a buffered aqueous solution of pH 2 to 13, more preferably of pH 5 to 9.

25 *Active ingredient (material of interest) (in the application media poorly soluble or insoluble material)*

Such active ingredient (material of interest) in said application media is poorly soluble or insoluble. Said active ingredient includes solids, liquids and gases. Preferably said active ingredient is a low molecular weight active ingredient or a

30 biopolymer (i.e. a polynucleotid or a protein). Active ingredients which are polymers obtained by emulsion polymerization are not within the scope of the present invention. More preferably, said active ingredient is selected from the group consisting of pharmaceuticals, crop protection agents, vitamins, dye stuffs,

organic pigments, inorganic pigments, fine chemicals, catalysts, enzymes, fillers, flame retarders, scale inhibitors, cosmetics, UV and light stabilizers.

Nanoparticular dispersion formulation

5   System comprising at least one continuous phase (dispersant), which is usually a liquid phase, and at least one dispersed phase. Said dispersed phase (further on called dispersed particles) may be a solid phase, a liquid phase or a gaseous phase (gas bubbles). In the context of the present invention said dispersed phase (dispersed particles) includes both, the active ingredient and the nanodispersant.

10   Therefore, said dispersion formulations may be emulsions, aerosols or suspensions. The average particle size, reported in terms of hydrodynamic radius (as explained in detail later in the specification), of the dispersed particles in said nanoparticular dispersion formulations is usually from 10 nm to 5 μm, preferably from 10 nm to 500 nm, more preferably from 20 nm to 50 nm.

15

The method of the present invention of making an array of n nanoparticular dispersion formulations comprises the following steps:

c)   making said array of n nanoparticular dispersion formulations by

20   c1)   a parallelized solid solution route, or

c2)   a parallelized general precipitation route, or

c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

25   wherein said active ingredient or combination of active ingredients is the same in each of said n nanoparticular dispersion formulations; and

wherein n is at least 2 and whererin at least one parameter selected from the group consisting of components employed, concentration of the components, temperature, reaction time, pH-value, other useful components and solvent, if

30   employed, is different in each of said nanoparticular dispersion formulations.

In a preferred embodiment of the present invention the method of making an array of n nanoparticular dispersion formulations comprises the following steps

a)   a method of making an array of m nanodipersants by a parallel polymerization process of at least 2 monomers A and B,

c)   making said array of n nanoparticular dispersion formulations wherein at least one of said obtained nanodispersants is employed by

    c1)   a parallelized solid solution route, or

    c2)   a parallelized general precipitation route, or

    c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

wherein either n or m is at least 2.

In a more preferred embodiment said method comprises the following steps

a)   a method of making an array of m nanodipersants by a parallel polymerization process of at least 2 monomers A and B,

b)   characterizing said nanodipersants,

c)   making said array of n nanoparticular dispersion formulations wherein at least one of said obtained nanodispersants is employed by

    c1)   a parallelized solid solution route, or

    c2)   a parallelized general precipitation route, or

    c3)   a parallelized reactive precipitation route,

d)   parallelized characterizing of said obtained n nanoparticular dispersion formulations,

wherein either n or m is least 2.

In preferred embodiments of the present invention n and m are, independent of each other, at least 8, more preferably at least 64, most preferably at least 8^4, and especially n and m are independent of each other at least 8^6.

5   The method of the present invention of how to find new nanodispersants (polymeric or oligomeric additives for nanoparticular formulations) can be divided in loops of the steps a) a method of making an array of m nanodispersants by a parallel polymerization process (design and synthesis of copolymer libraries), b) optionally characterizing said nanodispersants, c) making an array of n

10   nanoparticular dispersion formulations (preparation of formulations), d) parallelized characterizing of the obtained nanoparticular dispersion formulations (characterization of nanoparticular stability). Said steps a) to d) are preferably followed subsequently by step e) data mining for lead structures of (co)polymers and structure-property-relationships. With every loop, i.e. from primary to

15   secondary screening, the part of parameter space explored gets smaller and more thoroughly investigated. The number of loops is usually from 1 to 10. Depending on the respective dispersion formulation to be synthesized said number may be higher than 10.

20   Preferably, prior to step a) (the method of making an array of m nanodispersants) libraries of potentially suitable (co)polymers are designed.

Library design phase

In one embodiment, this invention provides useful methods for an integrated

25   combinatorial materials science research program for the discovery of novel nanodispersions for a particular active ingredient. Herein, the research program has the goal of creating a nanodispersion using a dispersant that will disperse a desired active ingredient in the desired media (e.g., typically water or a buffered aqueous solution). Thus, the active ingredient is limited to a single species or a

30   single combination of species, in this invention and the work-flow begins with the identification of the desired active ingredient. One or more combinatorial libraries of polymer nanodispersants are then designed for the chosen active ingredient. Existing library design software can be used for this design, such as

Library Studio™ (Symyx Technologies, Inc., Santa Clara, CA, USA) as disclosed in PCT/US99/24491 (published as WO 00/23921), which is incorporated herein by reference. The useful monomers for design of the nanodispersant are typically grouped into 4 categories, including hydrophobic, neutral hydrophilic, cationic

5   and anionic. These categories may be further sub-categorized by additional properties, for example, hydrophobic monomers may be sub-categorized into the monomers that provide the ability for π-stacking, large steric structures, etc. As discussed herein, and elsewhere (see e.g., PCT/US00/00418) the monomers for a nanodispersant library are typically prepared by parallel polymerization.

10

Time is saved because the computer program transfers a recipe file of automated calculated and cross-checked concentrations of ingredients e.g. monomers, initiators, control agents, additives, etc. into an instruction protocol for a robot. The robot fills the individual reaction chambers of parallel reactors (synthesis

15 regions) and controls the polymerization reaction (i.e. reaction time, temperature, etc.).

Step a)   A method of a making an array of m nanodispersants by a parallel
polymerization process of at least 2 monomers A and B

20

Preferably the polymerization process is a standard polymerization reaction in a solvent or in bulk selected from the group consisting of radical polymerization, cationic polymerization, anionic polymerization, polycondensation, polyaddition, polymer analogous reactions, living radical polymerization, homogeneously

25 catalyzed reactions.

Preferably said nanodispersants are prepared by polymerization of 2 to 6 different monomers, more preferably 2 to 5 different monomers and most preferably 2 or 4 different monomers. For example, adding complexity to a nanodispersant may be

30 desirable and this invention allows, for example, starting with three monomers and adding a fourth, etc.

The monomers A and B (and optionally further monomers C, D, ...) are preferably selected from the group consisting of hydrophobic, neutral hydrophilic, cationic and anionic monomers. Preferably, one of said monomers is a hydrophobic monomer and a second monomer is one of said monomers selected from the

5 group consisting of hydrophobic, neutral hydrophilic, cationic and anionic monomers. More preferably, one of said monomers is a hydrophobic monomer and a second monomer is a hydrophilic monomer which may comprise acidic or basic groups.

10 Suitable monomers are for example 1,4-cyclohexane dimethanol divinyl ether, 1,4-cyclohexane dimethanol monovinyl ether, 1-butene, 1-decene, 1-hexene, 1-octene, 1-pentene, 2-methyl-N-vinylimidazole, vinyl 4-tert.-butylbenzoate, acrolein, acrylamide, acrylonitrile, acrylic acid, allyl methacrylate, α-methylstryrene, butadiene, butanediol dimethacrylate, butanediol vinylether,

15 butanediol monoacrylate, butanediol monovinyl ether, butanediol methylmethacrylate, butylacrylate, butylmethacrylate, cyclohexyl vinyl ether, diethyleneglycole   divinyl ether, dimethylamino ethylacrylate, dimethylamino ethylacrylate-metochloride, dimethylamino ethylmethacrylate, dimethylamino ethylmethacrylate quaternized

20 by methylchloride, dimethylamino propylmethacrylamide, ethylene, ethyl acrylate, ethyldiglycol acrylate, ethyleneglycol dimethacrylate, ethyleneglycol monovinyl ether, ethylhexylacrylate, ethylmethacrylate, ethylvinyl ether, glycidylmethacrylate, hydroxyethyl methacrylate, hydroxypropyl methacrylate, isobutene, isobutylacrylate, isobutylmethacrylate, isoprene, maleic anhydride,

25 methacrylic acid, methacrylic acid anhydride, methylacrylate, methylenebisacrylic amide, methylmethacrylate, methylvinyl ether, n-butylvinyl ether, N-methyl-N-vinylacetamide, N-vinylcaprolactam, N-vinylimidazol, N-vinylpyrrolidone, octadecyl vinylic ether, phenoxyethylacrylate, propylene, styrene, tert.-butylacrylic amide, tert.-butylacrylate, tert.-butylmethacrylate, triethyleneglycol

30 dimethylacrylate, triethyleneglycol divinyl ether, triethyleneglycol divinylmethyl ether, trimethylol propane trimethacrylate, vinylacetate, vinylchloride, vinylformamide, vinylidenechloride, vinylisobutyl ether, N-vinylpiperidone, vinyl-(2-ethylhexyl) ether, vinylpropyl ether, vinylisopropyl ether, vinyldodecyl

ether, vinyl-tert.-butyl ether, hexanediol divinyl ether, hexanediol monovinyl ether, diethyleneglycol monovinyl ether, diethylamino ethylvinyl ether, polytetrahydrofurane-290-divinyl ether, tetraethyleneglycol divinyl ether, ethyleneglycol butylvinyl ether, ethyleneglycol divinyl ether, trimethylolpropane trivinyl ether and aminopropylvinyl ether.

Other components which are optionally employed in the polymerization process are for example initiators, catalysts, starters and modifiers.

In a preferred embodiment of the present invention the polymerization process is a radical polymerization to produce random radical (co)polymers. Preferred monomers A and B (and optionally further monomers C, D, ...) for use in the radical polymerization are non-polar monomers, for example

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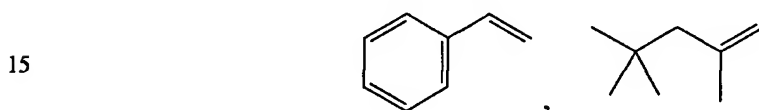
polar monomers comprising acidic groups, for example

, ,
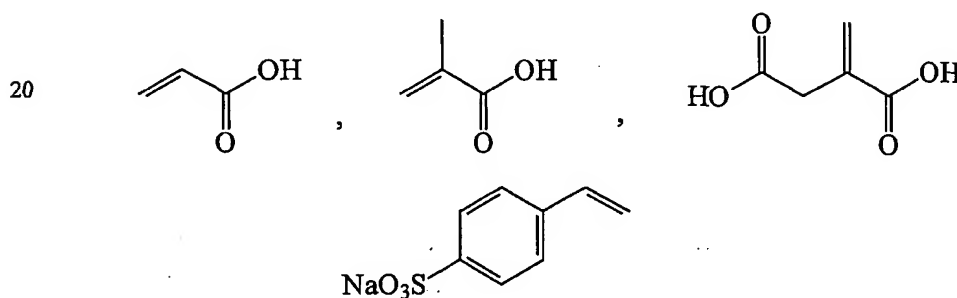
polar monomers comprising neutral groups, for example
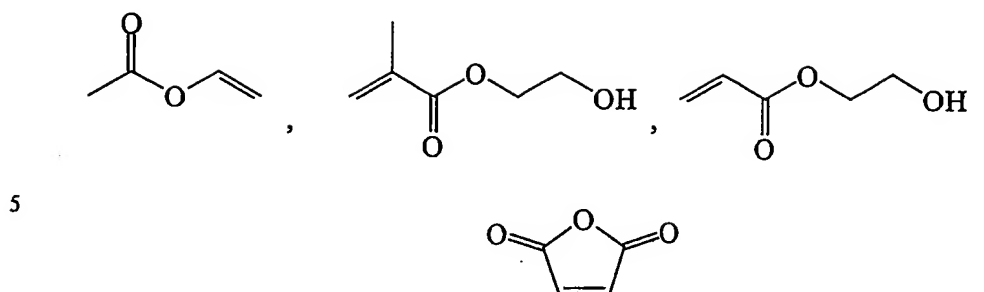
and polar monomers comprising basic groups, for example
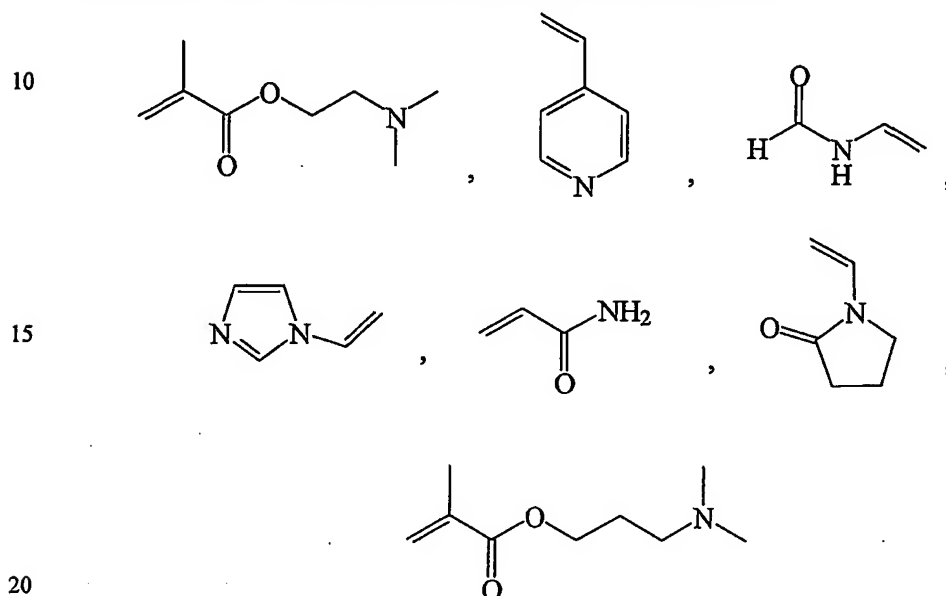
Suitable initiators which may be employed in said radical polymerization are polymerization initiators known in the art, for example hydrogen peroxide, inorganic persulfates, for example sodium peroxodisulfate, potassium peroxodisulfate and ammonium peroxodisulfate, and organic compounds like organic peroxides, peroxyesters, percarbonates and organic azo compounds. Suitable organic peroxides are for example diacetyl peroxide, dibenzyl peroxide, succinyl peroxide, di-tert.-butyl peroxide, tert.-butyl perbenzoate, tert.-butyl pivalate, tert.-butyl permaleinate, cumene hydroperoxide, diisopropyl peroxodicarbamate, bis-(o-toluolyl) peroxide, didecanoyl peroxide, dilauroyl peroxide, tert.-butyl hydroperoxide, tert.-butyl perisobutyrate, tert.-butyl peracetate, di-tert.-amyl peroxide, dibenzoyl · per-

oxide, tert.-butyl perpivalate, tert.-amyl perpivalate, tert.-butyl perneodecanoate and mixtures of said initiators. Suitable azo compounds are for example 2,2'-azo-bis-(2-amindinopropane) dihydrochloride, 2,2'-azo-bis-(N,N'-dimethylene) iso-butyramidine dihydrochloride, 2-(carbamoylazo)isobutyric nitril, 4,4'-azo-bis(4-cyanovaleric acid), 4,4'-dimethyl-2(phenylazo)-valeric nitril, 4-methoxy-2,4-dimethyl-2-(phenylazo)valeric nitril, 1,1'-azo-bis-(cyclohexane-1-carbonitril), 2,2'-azo-bis-(isobutyronitril), 2,2'-azo-bis-(2,4-dimethyl-valeric nitril), 2,2'-azo-bis-[2-(2-imidazoline-2-yl)propane] dihydrochloride, 2,2'-azo-bis-(2-methyl-butyric nitril) and/or 2,2'-azo-bis(isobutyrate).

Said peroxides may be employed in combination with reducing agents, for example Fe(SO$_4$), Na$_2$SO$_3$, NaHSO$_3$, sodium dithionate, triethanolamin and ascorbic acid. Additionally, the poymerization reaction may initiated by a photo initiator and irradiation with UV-light or by influence of high energy radiation.

If the polymerization reaction is conducted in an aqueous medium, preferably sodium or potassium peroxodisulfate are employed. If the polymerization reaction is conducted in bulk or in an organic solvent, preferably soluble initiators, for example organic peroxides, are employed.

Said initiators are employed in amounts known in the art, for example in amounts of 0.2 to 20 % by weight, preferably 1.0 to 10 % by weight, relating to the amount of monomers employed.

In a preferred embodiment of the present invention step a) is carried out in a solvent which is suitable to dissolve the monomers and the polymer. Preferred solvents are selected from the group consisting of aliphatic carboxylic acids with 1 to 3 carbon atoms, their amides, their mono-C$_1$-C$_4$-alkyl amides and di-C$_1$-C$_4$ alkyl amides, aliphatic and aromatic chlorohydrocarbons, alcohols of 1 to 5 carbon atoms, for example isopropanol, ketones of 3 to 6 carbon atoms, for

example acetone, aromatic hydrocarbons, N-alkylated lactams and mixtures of these.

Because of their good solvent power, preferred solvents are water, methanol, ethanol, isopropanol, formic acid, formamide, dimethylformamide, dimethylpropionamide, N-methylpyrrolidone, methylene chloride, chloroform, 1,2-dichloroethane, chlorobenzene, toluene, xylene, acetone, methylethylketone, methylisopropylketone, methylisobutylketone and mixtures of these.

Preferably, step a) comprises the steps

a1)   delivering said at least 2 monomers A and B, preferably a solvent, and optionally useful components appropriate for the employed polymerization process to each synthesis region on a substrate having k physically separate synthesis regions for m different nanodispersants,

a2)   subsequently or simultaneously reacting said monomers and optionally other useful components to form m different nanodispersants,

a3)   optionally delivering said other components deferred to each synthesis region,

wherein at least one parameter selected from the group consisting of monomers or other components employed, concentration of said monomers or said useful components, solvent employed, temperature, reaction time is different in each of said m nanodispersions.

l and m are independent of each other at least 2 with the proviso that k is $\geq$ 2. In a preferred embodiment of the present invention k and m are at least 8, more preferably at least 64, most preferably at least 8^4, and especially n and m are independent of each other at least 8^6. It is also possible that the number k of said physically separate synthesis regions on the substrate is larger than the number m of said nanodispersants. This means that not all of the physically separate synthesis regions on the substrate are filled up in order to leave blanks or standards.

Preferably, said physically separate synthesis regions on said substrate are wells on a microtiter plate reactor or vials of a parallel reactor. A parallel reactor which may be used in the present invention is described for example in WO 00/09255,

5   which is incorporated herein by reference.

The volume of said wells or vials is usually at most 100 ml, preferably at most 10 ml, more preferably at most 1 ml and most preferably from 200 to 1000 µl.

10   Said parallel polymerization to produce said nanodispersants of the present invention provides a method to produce a high number of nanodispersants which can be synthesized in one day. Generally, said parallel polymerization process provides a process for the production of 100 to 100,000, preferably more than 1000 to 100,000, more preferably 10,000 to 100,000 nanodispersants a day. Using

15   for example an array of several microtiter plate reactors of 96 wells of up to 1000 µl volume each, increases the number of nanodispersants synthesized to more than 1000 a day.

Said at least 2 monomers A and B, said solvent and optionally said other

20   components appropriate for the employed polymerization process can be delivered to said synthesis regions on said substrate by a method for delivery of reactant components as mentioned above. Preferably, said at least two monomers A and B, said solvent and said other useful components appropriate for the employed polymerization process are delivered to said synthesis regions on said

25   substrate from a pipette by automated and/or parallel delivering. In another embodiment of the present invention the components are delivered to said synthesis regions on said substrate from an ink-jet dispenser selected from the group consisting of a pulse pressure ink-jet dispenser, a bubble-jet ink-jet dispenser and a slit jet ink-jet dispenser.

30

The moving of the dispenser with respect to the substrate is realized as mentioned above.

In a preferred embodiment of the present invention the delivering of said at least 2 monomers A and B, optionally a solvent and optionally other components appropriate for the employed polymerization process to each synthesis region on a substrate having k physically separate synthesis regions for m different nanodispersants (step a1) comprises the following steps:

a11)   identifying a reference point on said substrate,

a12)   moving a dispenser of said at least 2 monomers A and B, optionally said solvent or optionally said other useful components a fixed distance and direction from said reference point such that said dispenser is positioned approximately above a first synthesis region on said substrate,

a13)   delivering one of said at least 2 monomers A and B, optionally said solvent or optionally said other components to said first synthesis region, and

a14)   repeating steps a12) and a13) for each remaining component consisting of said at least 2 monomers A and B, optionally said solvent and optionally said other components,

wherein k and m are independent of each other at least 2 with the proviso that $k \geq 2$.

The array of polymer dispersants can be cleaned, washed or otherwise treated as those of skill in the art are aware. The array of polymer dispersants may be used one or more times to form a nanodispersion array or library using the different routes as discussed herein.

Step b)   Optionally characterizing said nanodispersants

The nanodispersants in the array can be screened to determine if the desired material was prepared (for example through testing a random selection of the nanodispersants or "spot" testing or more complete testing) in a manner known in the art, such as using rapid chromatography techniques (see, e.g., PCT/US99/07304).

The nanodispersants in the array can be screened for example by extended GPC (gel permeation chromatography), accelerated GPC, rapid GPC, rapid gradient HPLC (high pressure liquid chromatography), flow injection analysis or NMR (nuclear magnetic resonance spectroscopy), GC (gas chromatography) or IR

5   (infrared spectroscopy).

In a preferred embodiment of the present invention the nanodispersants obtained are characterized for molecular weight (Mw) and monomer conversion by rapid GPC.

10

Said characterization of the nanodispersants in step b) is useful to optimize the conditions of the synthesis of said nanodispersants prior to the screening process. It is also very useful to characterize the most promising leads and hits identified by the screening process.

15

Step c)   Method   of   making   an   array   of   n   nanoparticular   dispersion formulations, by

     c1) a parallelized solid solution route, or
20   c2) a parallelized general precipitation route, or
     c3) a parallelized reactive precipitation route.

c1) Parallelized solid solution route

In a first step, a solution of at least one of said nanodispersants and a solution of
25   one active ingredient or a combination of more than one active ingredients are mixed in the same or in compatible solvents in similar parallel geometries, i.e. microtiter plates. The mixing process can be assisted by moderate stirring or vortexing. In a second step, the solvent will be evaporated e.g. by vacuum under simultaneous IR irradiation. The molecular disperse solid solution formed by this
30   process can be analyzed by X-ray diffraction (missing Bragg peaks), DSC (differential scanning calorimetry) or optical transmission. In a third step, the redispersion process is triggered by adding a non-solvent of the active ingredient, e.g. water, also potentially assisted by moderate stirring, vortexing or ultrasonic

treatment. A nanoparticular formulation is made by this redispersion process in a successful case.

In a preferred embodiment of the present invention the parallelized solid solution route of step c1) comprises the following steps:

c11)   delivering a solution of at least one of said nanodispersants and a solution of an active ingredient to each synthesis region on a substrate having k physically separate synthesis regions,

c12)   forming n solid solutions of said at least one nanodispersant and said active ingredient,

c13)   dispensing said at least one application media into said k physically separate synthesis regions on said substrate, and optionally

c14)   agitating the obtained mixture to form n different nanoparticular dispersion formulations,

wherein k and n are independent of each other at least 2 with the proviso that k ≥ 2.

Said nanodispersants as well as said active ingredient may be delivered to each synthesis region for example in form of their solutions, suspensions, emulsions, dispersions or in bulk or gaseous form, depending on their physical properties.

The solvents to dissolve said at least one nanodispersant and said active ingredient are depending on the nature of the active ingredient and the nanodispersant. Suitable solvents are for example selected from the group consisting of aliphatic carboxylic acids with 1 to 3 carbon atoms, their amides, their mono-C_1-C_4-alkyl amides and di-C_1-C_4 alkyl amides, aliphatic and aromatic chlorohydrocarbons, alcohols of 1 to 5 carbon atoms, for example isopropanol, ketones of 3 to 6 carbon atoms, for example acetone, aromatic hydrocarbons, N-alkylated lactams and mixtures of these. Because of their good solvent power, preferred solvents are methanol, ethanol, isopropanol, formic acid, formamide, dimethylformamide, dimethylpropionamide, N-methylpyrrolidone, methylene chloride, chloroform,

1,2-dichloroethane, chlorobenzene, toluene, xylene, acetone, methylethylketone, methylisopropylketone, methylisobutylketone and mixtures of these.

The solid solutions are formed by evaporating the solvent e.g. by a vacuum under simultaneous IR irradiation. Preferably, said solid solutions are formed by spray drying, vacuum drying or lyophilization or other drying techniques, for example by circulating warm dry air across the samples at ambient pressure.

The step of delivering at least one of said nanodispersants and said active ingredient (c11) to the synthesis regions on the substrate can be realized as mentioned above. In a preferred embodiment of the present invention said step of delivering said components comprises the following steps:

c111)   identifying a reference point on said substrate,

c112)   moving a dispenser of said at least one nanodispersant and said active ingredient a fixed distance and direction from said reference point such that said dispenser is positioned approximately above a first synthesis region on said substrate,

c113)   delivering one of said at least one nanodispersants or said active ingredient to said first synthesis region, and

c114)   repeating steps c112) and c113) for the remaining component(s) consisting of said at least one nanodispersant and said active ingredient.

The at least one nanodispersant and the active ingredient can be delivered to each synthesis region on said substrate by a method for delivery of reactant components as mentioned above. Preferably, said at least one nanodispersant and said active ingredient are delivered to said synthesis regions on said substrate from a pipette by automated and/or parallel delivering. In another embodiment of the present invention said components are delivered by an ink-jet dispenser selected from the group consisting of a pulse pressure ink-jet dispenser, a bubble-jet ink-jet dispenser and a slit jet ink-jet dispenser.

c2) Parallelized general precipitation route

The solution of the at least one nanodispersant in the application media and the active ingredient are mixed in a way that for the resulting mixture of solvents the saturation concentration of the active ingredient will be exceeded. This ends up in a spontaneous formation of nanoparticular structures. In this process the state of aggregation of the active ingredient will be changed, not its chemical identity. This can be achieved by two chemically different solvents or with the same solvent by changing e.g. pH value, ionic strength or temperature. The process conditions can vary in this parallel precipitation, e.g. droplet dispensing or injection of the solution of the at least one active ingredient with a parallel robot to the solution of the at least one nanodispersant or a mixing chamber with defined flow rates and contact times between the solutions of the at least one nanodispersant and the active ingredient.

In a preferred embodiment of the present invention the parallelized general precipitation route of step c2) comprises the following step

c21) delivering a first solution of at least one of said application media and a second solution of said active ingredient with said first and/or second solution additionally comprising said at least one nanodispersant, to each synthesis region on a substrate having k physically separate synthesis regions,

wherein said solvents and said solutions are miscible and chosen in a way that for the resulting mixture of said solvents the saturation concentration of said active ingredient will be exceeded to form n different nanoparticular dispersion formulations.

The solvents are depending of the nature of the active ingredient and the nanodispersant. Suitable solvents are the same as mentioned under c1).

Preferably, said physically separate synthesis regions on said substrate are wells on a microtiter plate reactor or vials of a parallel reactor.

Suitable methods for delivery of reactant components are already mentioned above. Preferred methods for the delivery of reactant components are the same methods as mentioned under c1).

5   Suitable methods for moving the dispenser with respect to the substrate are mentioned above. In a preferred embodiment of the present invention the step of delivering a solution of at least one of said nanodispersants and a solution of said active ingredient (c21) each comprises the following steps:

10   c211)   identifying a reference point on said substrate,

c212)   moving a dispenser of said solution of said at least one nanodispersant and said solution of said active ingredient a fixed distance and direction from said reference point such that said dispenser is positioned approximately above a first synthesis region on said substrate,

15   c213)   delivering one of said solution of said at least one nanodispersant or said solution of said active ingredient to said first synthesis region, and

c214)   repeating steps c212) and c213) for the remaining component(s) consisting of said solution of said at least one nanodispersant and said solution of said active ingredient.

20

c3) Parallelized reactive precipitation route

Reactive precursors of the active ingredient can be stabilized by at least one nanodispersant in a way that the reaction resulting in the final substrate occurs in nanoparticles of a nanoparticular dispersion formulation. Alternatively, first the

25   reaction of one or more precursors of the active ingredient producing the final active ingredient is finished and afterwards the at least one nanodispersant stabilizes the substrate in form of nanoparticles of a nanoparticular dispersion formulation. Process conditions are similar as described in c2). Reactions can be chemical reactions, salt formations, or complexations. Active ingredients which

30   are polymers obtained by emulsion polymerization are not within the scope of the present invention. In a preferred embodiment, c3), the parallelized reactive precipitation route (c3) comprises the following steps

145

c31)   delivering a solution of one or more reactive precursors of said active ingredient and a solution of said at least one nanodispersant in the application media to each synthesis region on a substrate having k physically separate synthesis regions,

5   c32)   reacting said one or more precursors to produce said active ingredient which is stabilized by said at least one nanodispersant to form n different nanoparticular dispersion formulations,

wherein k and n are independent of each other at least 2 with the proviso that k ≥ n.

10

If more than one reactive precursor for the active ingredient is employed, the nanodispersant and the reactive precursors may be delivered to each synthesis region in any order. Suitable reactive precursors are for example $CaCl_2 + Na_2CO_3$, $BaCl_2 + Na_2SO_4$, $TiCl_4 + NaOH$ and $CaCl_2 + Na_2C_2O_4$. Active ingredients which

15   are polymers obtained by emulsion polymerization are not within the scope of the present invention.

Preferably, said physically separate synthesis regions on said substrate are wells on a microtiter plate reactor or vials of a parallel reactor.

20

The at least one nanodispersant and the precursor of said active ingredient are delivered to the synthesis regions on the substrate by methods for delivery of reactant components as mentioned above. Preferred methods for the delivery of reactant components are the same methods as mentioned under c1).

25

The solvents employed are depending of the nature of the active ingredient and the nanodispersant. Suitable solvents are the same as mentioned under c1).

The components are deposited to each synthesis region on a substrate having k

30   physically separate synthesis regions by moving the dispenser with respect to the substrate as mentioned above. Preferably, the step of delivering a solution of a reactive precursor of said active ingredient and a solution of said at least one nanodispersant each comprises the following steps

c311) identifying a reference point on said substrate,

c312) moving a dispenser of said solution of a reactive precursor of said active ingredient and said solution of said at least one nanodispersant a fixed distance and direction from said reference point such that said dispenser is positioned approximately above a first synthesis region on said substrate,

c313) delivering one of said solution of a reactive precursor of said active ingredient or said solution of said at least one nanodispersant to said first synthesis region, and

c314) repeating steps c312) and c313) for the remaining component(s) consisting of said solution of a reactive precursor of said active ingredient and said solution of said at least one nanodispersant.

Said parallel preparation to produce said nanoparticular dispersion formulations of the present invention provides a method to produce a high number of nanoparticular dispersion formulations which can be synthesized in one day. Generally, said parallel preparation process provides a process for the production of 100 to 100,000, preferably more than 1000 to 100,000, more preferably 10,000 to 100,000 of nanoparticular dispersion formulations a day.

d) *Parallelized characterizing of said obtained n nanoparticular dispersion formulations*

Generally, this invention utilizes a method for screening the results of the formulation work described above. The general route is particularly important in combinatorial materials science where an object is to form vast arrays of different formulations for testing in different applications so that a combinatorial materials science program for nanodispersion formulation can be effectively implemented using the methods of this invention. For example, initially a large compositional space for the nanodispersants may be rapidly explored through the preparation of compositional gradients within a binary (A,B) or ternary composition space of monomers (A, B, and C) or composition spaces of orders higher than 3. Such a composition space may be studied through the creation of a matrix of ratios

containing 0-100% for each monomer. After a first array is prepared and screened, a focused second array limited to a compositional range of interest may be examined with a finer gradient. This process can continue as long as the screening can meaningfully distinguish between neighboring array elements.

5

In one embodiment, this invention provides useful methods for an integrated combinatorial materials science research program for the discovery of novel nanodispersions for a particular active ingredient. Herein, the research program has the goal of creating a nanodispersion using a dispersant that will disperse a

10   desired active ingredient in the desired media (e.g., typically water or a buffered aqueous solution). The work-flow begins with the identification of the desired active ingredient. One or more combinatorial arrays of polymer nanodispersants are then designed for the chosen active ingredient. Existing library design software can be used for this design, such as Library Studio™ (Symyx

15   Technologies, Inc., Santa Clara, CA, USA) as disclosed in PCT/US99/24491 (published as WO 00/23921), which is incorporated herein by reference.

The useful monomers for design of the nanodispersant are typically grouped into 4 categories, including hydrophobic, neutral hydrophilic, cationic and anionic. These categories may be further sub-categorized by additional properties, for

20   example, hydrophobic monomers may be sub-categorized into the monomers that provide the ability for π-stacking, large steric structures, etc. As discussed herein, and elsewhere (see e.g., PCT/US00/00418) the monomers for a nanodispersant array are typically prepared by parallel polymerization. The nanodispersants in the array can be screened to determine if the desired material was prepared (for

25   example through testing a random selection of the nanodispersants or "spot" testing or more complete testing) in a manner known in the art, such as using rapid chromatography techniques (see, e.g., PCT/US99/07304). The array of polymer dispersants may be used one or more times to form a nanodispersion array using the different routes as discussed herein. In some embodiments, the

30   quantity of dispersant is large enough to be used for several preparations of nanodispersant arrays, for example, through daughtering the dispersant array into or onto several substrates (such as microtiter plates having wells). The

preparation of the arrays or libraries of nanodispersions proceeds through one of the routes discussed in detail above, including the solid solution route, the general precipitation route and the reactive precipitation route.

Afterwards, the arrays of nanodispersions are "screened" for a property of interest.

5   As used herein, a "screen" is a test performed on one or more members of a library of materials to determine if a property or compound of interest is present. A screen may be simple and provide only minimal information or data or may be more complex and provide complex information or data. Generally, screening is divided up into levels depending on the type and amount of information that is

10 provided. The first level is a primary screen, which is the fastest or highest throughput screening level. A primary screen, because of the need for fast rates, should be able to screen arrays at a pace that accommodates the method of array preparation. In other words, the primary screen should be able to screen in a day the nanodispersion arrays that are formulated in a day, with the formulation rate

15 begin adjusted to accommodate screening and/or vice versa. In the context of this invention, a primary screen will test and determine at least one property or compound in the members of the array. A primary screen here will typically be either a parallel visual screen, parallel optical screen or high throughput chromatography, which parallel visual or optical screening being preferred.

20 The next level is secondary screening, which is testing to provide more information on array members that pass the primary screen. In some embodiments, secondary screening will require preparation of larger quantities of the nanodispersions that pass the primary screen and in other embodiments, the secondary screen will be performed on arrays that have been created from those

25 members of arrays that have passed the primary screen (e.g., an array constructed only of members from previous libraries). Since secondary screening typically is performed on fewer samples than primary screening, the secondary screen may typically take longer than the primary screen and provides additional data. Thus, the secondary screen can be chosen from the same list of screens discussed herein,

30 but may also include a stability test, melting point tests (e.g., such as differential scanning calorimetry), pH testing, chromatography (e.g., size exclusion chromatography), spectroscopy (e.g., UV-VIS absorption), dynamic light

scattering for measuring the average particle size, for example FOQELS and FODLS (Fiber Optic Dynamic Light Scattering) or another test to determine composition of matter or another test as is known to those of skill in the nanodispersion art.

5

For example, stability screening can be performed by observing the stability of the nanodispersion array member(s) at a first time and possibly a second time and determining any change in nanodispersion stability. Typically, stability is determined by testing the size of the dispersed phase (e.g., by light scattering) or

10   the composition of the nanodispersion (e.g., by chromatography) or visually to observe whether a nanodispersion has formed. The first time will be near to time of nanodispersion formation and can range from 2 minutes to as much as 48 hours, with less than 24 hours being preferred. Stability at a first time may be a typical primary screen, e.g., it determines if a nanodispersion has formed at all.

15   The second time for stability determination can be at about 24 hours from the time of nanodispersion formation to as much as 1, 2, 3 or 4 weeks from the time of formation. This provides longer term stability testing. Another option is aging of the nanodispersions between two or more stability tests. Aging of the nanodispersions can be accomplished through thermocycling, shearing, shaking or

20   stirring the formulations. Thermocycling is preferred, with the temperature for cycling varying from room temperature to up to 75°C, and preferably at least about 40°C.

After primary and secondary screening, focussed arrays from within the

25   compositional regions shown to have the most desirable results from the screening are prepared. In addition, bulk samples of identical compositions were prepared according to conventional methods for the purpose of scale-up, structural and/or composition comparisons. Thus, it can be seen how those of skill in this art can effectively utilize the methods of this invention for a combinatorial materials

30   science research program.

e)   Data-mining for lead structures

Preferably, subsequent to the steps a) to d) for the preparation and characterization of an array of nanoparticular dispersion formulations a data-mining for lead structures of nanodispersants and formulation-processing-property-relationships is

5   carried out. Starting from lots of recipes, preparation conditions, and stabilization characterization of such formulation-processing-property-relationships can be generated by numerical procedures, e.g. fuzzy logic or neuronal networks. This allows a higher level screening (next loop) in a promising region of the parameter space, e.g., a more detailed investigation of a ternary composition diagram of

10   monomers using smaller steps in the variation of monomer composition or a slight variation of the preparation conditions, i.e. temperature of injection. This higher level screening loops can be refined, as long the stabilization properties will become better.

15   The average particle size, reported in terms of hydrodynamic radius, of the dispersed particles in said nanoparticular dispersion formulations is usually from 10 nm to 5 μm, preferably from 10 to 500 nm, more preferably from 20 to 50 nm. The average particle sizes were characterized by light scattering methods, preferably Fiber Optic DLS measurements (FODLS). Therefore, samples of said

20   dispersion formulations were diluted to approximately 0.005% solids in an appropriate carrier aqueous solution. Average particle sizes were determined by second order cumulant analysis and are reported in terms of hydrodynamic radius (r_H).

25   Another embodiment of the present invention is an array of at least 8 different nanoparticular dispersion formulations on a substrate at known locations thereon, with said nanoparticular dispersion formulations containing

        -   at least one nanodispersant,
30   -   at least one application media, and
        -   an active ingredient
        wherein said active ingredient is the same in each of said nanoparticular dispersion formulations .

Said at least one nanodispersant, at least one application media and said active ingredient are already defined above. Preferably, the array has at least 48, more preferably at least 64, most preferably at least 8^4 different nanoparticular dispersion formulations.

Said nanoparticular dispersion formulations are of interest because of their very specific advantages, e.g. coloristic, rheological, bioavailability, non-linear optical properties or other properties.

Another embodiment of the present invention is a method of making an array of m nanodispersants by a parallel polymerization process comprising the steps

- delivering at least 2 monomers A and B, optionally a solvent and optionally other components appropriate for the employed polymerization process, e.g. initiators, catalysts, starters, modifiers to each synthesis region on a substrate having k physically separate synthesis regions for m different nanodispersants,

- subsequently or simultaneously reacting said monomers or other useful components to form m different nanodispersants,

wherein at least one parameter selected from the group consisting of monomers or other components employed, concentration of said monomers or said other useful components, solvent employed, temperature reaction time is different in each of said m nanodispersants and k and m are independent of each other at least 2 with the proviso that $k \geq m$.

The monomers, solvent, other components appropriate for the employed polymerization process and the polymerization process are already mentioned above.

Said nanodispersants can be employed in a method of making an array of n nanoparticular dispersion formulations comprising the steps c) and d) of the

method of making an array of n nanoparticular dispersion formulations as mentioned above.

By said method of making an array of nanodispersants, new effective nanodispersants can be discovered in a short development time.

This method provides an array of at least 8 different nanodispersants on a substrate at known locations thereon, wherein said nanodispersants are synthesized by polymerization of at least 2 monomers A and B, wherein one of said monomers is a hydophilic monomer and the other monomer is a hydrophobic monomer.

Suitable monomers and polymerization processes are already mentioned above under a).

Another embodiment of the present invention is a method of making an array of n solid solutions by a parallelized solid solution route comprising the following steps

• delivering at least one of said nanodipersants as mentioned above and a solution of an active ingredient to each synthesis region on a substrate having k physically separate synthesis regions;

• forming n solid solutions of said at least one nanodipersant and said active ingredient,

wherein said active ingredient is the same in each of said n solid solutions, and

wherein k and n are independent of each other at least 2 with the proviso that k ≥ 2.

The steps of delivering the components to said synthesis regions on said substrate and for forming said solid solutions are already mentioned above.

Said method of making an array of solid solutions provides an array of at least 8 different solid solutions on a substrate at known locations thereon, wherein said solid solutions containing

o   at least one nanodispersant, and

5   o   an active ingredient,

wherein said active ingredient is the same in each of said solid solutions.

Said at least one nanodispersant and said active ingredient are already mentioned above.

10

The present invention provides a method for discovering new effective nanodispersants and new dispersion formulations in a very short time.

15   **Examples**

1.   **Random radical (co)polymers**

Two embodiments of the (co)polymer dispersing agents (nanodispersants) are shown below (libraries 1 and 2).

20

Library 1 is a (co)polymer library comprising styrene (S) / acrylic acid (AA) / dimethylaminoethylmethacrylate (DMAEM). The composition parameter space includes up to 100% of each monomer. It was designed to have the following compositional makeup:

25

Library 1: composition (S/AA/DMAEM [mol% of feed])

	1	2	3	4	5	6	7	8	9	10	11	12
A	95/05 /00	90/09 /01	70/26 /04	80/15 /05	85/10 /05	100/0 /0	90/05 /05	85/05 /10	80/05 /15	70/05 /25	90/00 /10	95/00 /05
B	80/20 /00	75/24 /01	50/43 /07	60/30 /10	70/19 /11	75/14 /11	60/18 /22	70/11 /19	60/10 /30	50/08 /42	75/01 /24	80/00 /20
C	65/35 /00	60/38 /02	35/55 /10	40/45 /15	50/32 /18	55/25 /20	45/25 /30	50/18 /32	40/15 /45	35/10 /55	60/02 /38	65/00 /35

D	50/50 /00	45/52 /03	25/64 /11	30/53 /18	35/42 /23	40/33 /27	30/31 /38	35/23 /42	30/17 /53	25/11 /64	45/03 /52	50/00 /50
E	35/65 /00	30/67 /03	15/72 /13	20/60 /20	25/49 /26	25/41 /34	20/36 /44	25/26 /49	20/20 /60	15/13 /72	30/03 /67	35/00 /65
F	20/80 /00	15/81 /04	10/77 /13	10/68 /22	15/55 /30	15/47 /38	10/40 /50	15/30 /55	10/22 /68	10/14 /76	15/04 /81	20/00 /80
G	05/95 /00	05/90 /05	05/81 /14	05/71 /24	05/62 /33	05/52 /43	05/43 /52	05/33 /62	05/24 /71	05/14 /81	05/05 /90	05/00 /95
H	0/100 /0	00/95 /05	00/85 /15	00/75 /25	00/65 /35	00/55 /45	00/45 /55	00/35 /65	00/25 /75	00/15 /85	00/05 /95	0/0/1 00

These (co)polymers were found to have the following molecular weights when compared to polystyrene standards. The (co)polymers were dissolved to 5.0 mg/ml in dimethylformamide (DMF) with 0.1% trifluoroacetic acid (TFA) (DMF-0.1% TFA). Using an accelerated GPC protocol with an evaporative light scattering detector (8 minutes / sample), the following polystyrene equivalent values of Mw (weight average molecular weight) were found:

Mw of library 1: Mw [Dalton x 1000]

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	20	74	76	75	8	73	91	100	113	90	71
B	16	36	96	100	98	96	117	112	124	132	113	109
C	19	52	108	119	115	118	131	128	137	140	127	123
D	24	66	122	126	131	128	140	143	146	148	139	135
E	27	76	120	136	135	141	148	144	149	155	146	143
F	28	82	122	113	154	153	164	153	163	162	158	154
G	33	87	52	58	216	203	188	181	173	164	172	169
H	50	154	311	403	434	334	255	221	203	195	196	182

Composition and conversion were checked using ^{1}H-NMR. Spot checks of composition were performed across the library. The following residual monomer was found (wt% monomer/wt% total polymer x 100):

Conversion of library 1: residual monomer (S/AA/DMAEM [wt%]) – spot checked by ^{1}H-NMR

	1	2	3	4	5	6	7	8	9	10	11	12
A						00/00 /00						
B							00/07 /01					
C												
D	00/06 /00											00/00 /01
E					00/12 /01			00/07 /01				
F												
G												
H	00/02 /00					00/--- /00						00/00 /01

Library 2 is another (co)polymer dispersing agent (nanodispersant) embodiment and is a (co)polymer library comprising the full ternary compositional space of styrene (S) / acrylic acid (AA) / 4-vinyl pyridine (4-VP). It was designed to have the following compositional makeup:

Library 2: composition (S/AA/4-VP [mol% of feed])

	1	2	3	4	5	6	7	8	9	10	11	12
A	95/05 /00	90/09 /01	70/26 /04	80/15 /05	85/10 /05	100/0 /0	90/05 /05	85/05 /10	80/05 /15	70/05 /25	90/00 /10	95/00 /05
B	80/20 /00	75/24 /01	50/43 /07	60/30 /10	70/19 /11	75/14 /11	60/18 /22	70/11 /19	60/10 /30	50/08 /42	75/01 /24	80/00 /20
C	65/35 /00	60/38 /02	35/55 /10	40/45 /15	50/32 /18	55/25 /20	45/25 /30	50/18 /32	40/15 /45	35/10 /55	60/02 /38	65/00 /35
D	50/50 /00	45/52 /03	25/64 /11	30/53 /18	35/42 /23	40/33 /27	30/31 /38	35/23 /42	30/17 /53	25/11 /64	45/03 /52	50/00 /50
E	35/65 /00	30/67 /03	15/72 /13	20/60 /20	25/49 /26	25/41 /34	20/36 /44	25/26 /49	20/20 /60	15/13 /72	30/03 /67	35/00 /65
F	20/80 /00	15/81 /04	10/77 /13	10/68 /22	15/55 /30	15/47 /38	10/40 /50	15/30 /55	10/22 /68	10/14 /76	15/04 /81	20/00 /80
G	05/95 /00	05/90 /05	05/81 /14	05/71 /24	05/62 /33	05/52 /43	05/43 /52	05/33 /62	05/24 /71	05/14 /81	05/05 /90	05/00 /95

H	0/100/0	00/95/05	00/85/15	00/75/25	00/65/35	00/55/45	00/45/55	00/35/65	00/25/75	00/15/85	00/05/95	0/0/100

These (co)polymers were found to have the following molecular weights when compared to polystyrene standards. The (co)polymers were dissolved to 5.0 mg/ml in dimethylformamide (DMF) with 0.1% trifluoroacetic acid (TFA) (DMF-0.1% TFA). Using an accelerated GPC protocol with an evaporative light scattering detector (8 minutes / sample), the following polystyrene equivalent values of Mw (weight average molecular weight) were found:

Mw of library 2: Mw [Dalton x 1000]

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	17.4	56	56	56	8	53	69	81	96	69	50
B	15	28.5	65	74	74	74	91	87	101	111	94	90
C	19	38	74	81	84	90	101	100	104	107	105	102
D	23	47	75	85	86	93	99	104	107	113	114	111
E	26	55	70	78.39	86	93	97	102	110	113	114	118
F	28	54.8	65	71	80	87	91	99	103	110	112	116
G	32	43	57	68	71	78	86	96	106	112	116	120
H	35	40	45	57	64	69	81	92	x[1]	x	x	x

[1] no data

Composition and conversion were checked using ^{1}H-NMR. Spot checks of composition were performed across the library. The following residual monomer was found (wt% monomer/wt% total polymer x 100):

Conversion of library 2: residual monomer (S/AA/4-VP [wt%]) – spot checked by ^{1}H-NMR

	1	2	3	4	5	6	7	8	9	10	11	12
A						00/00/00						
B							00/05/01					
C												

	1	2	3	4	5	6	7	8	9	10	11	12
D	00/06 /00											00/00 /01
E					00/04 /00		00/06 /01					
F												
G												
H	00/02 /00				00/16 /00							00/00 /01

(Co)polymer synthesis

In the preferred synthesis embodiment, (co)polymer synthesis was carried out under argon environment in 1 ml glass vials. A reaction block of 96 vials was loaded robotically at room temperature with monomer, solvent, and initiator:

|	
Total volume of reaction solution:	700 µl
Amount of monomer added:	10% by weight
Solvent used:	dimethylformamide (DMF)
Initiator used and amount added:	azobisisobutyronitrile (AIBN), 2.0 mol% to total monomer

After loading, the reaction block was sealed and heated to 60 to 80°C, preferably to 70°C under stirring with magnetic stirring bars. In an alternative embodiment, a parallel condensing mantle is placed over the top of the reactor block so that the reactions can be run at ambient pressure. The reaction was run for 4 to 8 hours, preferably 6 hours before it was allowed to cool to ambient temperature.

2. Dispersion formulations and formulation process

Dispersions of a dye substrate of the formula I (Palanil© Brilliantrot BEL) were formed by dispersing a preformed solid solution of the (co)polymers (nanodispersants) and the dye substrate of the formula I (active ingredient).

(I)
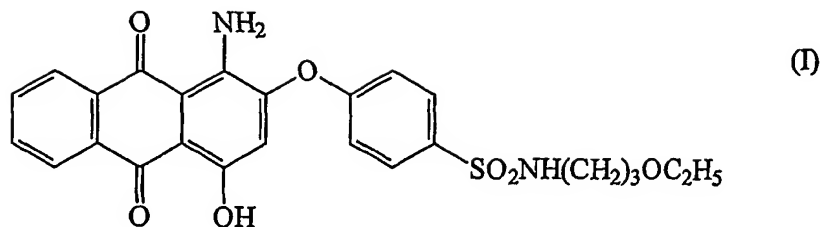
Formation of the solid solution

Typically solid solutions are formed in a spray drying process. In the process embodiment disclosed in the examples of the present invention arrays of parallel solid solutions were formed by drying solutions of the (co)polymer (nanodispersant) and the dye substrate (active ingredient) in glass vials to form thin films of intimately mixed solid solutions of (co)polymer (nanodispersant) and dye substrate (active ingredient). The preferred process to form solid solutions comprises making a stock solution of both the (co)polymer and the dye substrate in DMF for each dye : (co)polymer ratio of interest. The stock solutions were agitated for 5 minutes using a bench-top ultrasonic mixer and appropriate amounts of each solution were dispensed into glass vials in order to provide the desired amounts of total solids for a 500 µl dispersion of each formulation. Plates of vials were then placed in a parallel spin-vac dryer. They were dried for 2 to 14 hours, preferably for 8 hours at 1.0 torr with mild heating (40°C for the first 15 minutes). After drying, vials were left with a film of intimately mixed (co)polymer and the dye substrate of the formula I (Palanil© Brilliantrot BEL).

The application media was added to each well of the array of solid solutions, and thus a formulation was created from each array element. In addition, the residual monomer was mesured for only selected wells because NMR was used. In the following certain, representative formulations are described.

Stable nanoparticular dispersion formulations

Formulation 1:

(Co)polymer: Library 1, well C5 (S/AA/DMAEM = 50/32/18 mol%)

Residual monomer by ^{1}H-NMR: 00/12/02 (S/AA/DMAEM wt%)

Aqueous system: 500 µl buffered solution of pH = 9 (boric
                                   acid 0.05 M and potassium chloride 0.05 M)
Total solids content: 0.1 wt% solids
wt dye/wt total solids: 20 wt%

5

Formulation 2:
(Co)polymer: Library 1, well C8 (S/AA/DMAEM =
                                   50/18/32 mol%)
Residual monomer by ^{1}H-NMR: 00/07/04 (S/AA/DMAEM wt%)
10   Aqueous system: 500 µl of pure water
Total solids content: 5.0 wt% solids
wt dye/wt total solids: 10 wt%

ı   *Formulation 3:*
15   (Co)polymer: Library 1, well D9 (S/AA/DMAEM =
                                   30/17/53 mol%)
Residual monomer by ^{1}H-NMR: 00/04/03 (S/AA/DMAEM wt%)
Aqueous system: 500 µl of pure water
Total solids content: 5.0 wt% solids
20   wt dye/wt total solids: 10 wt%

Formulation 4:
(Co)polymer: Library 2, well D8 (S/AA/4-VP = 35/23/42
                                   mol%)
25   Residual monomer by ^{1}H-NMR: 00/06/02 (S/AA/4-VP wt%)
Aqueous system: 500 µl buffered solution of pH = 9 (boric
                                   acid 0.05 M and potassium chloride 0.05 M)
Total solids content: 0.5 wt% solids
wt dye/wt total solids: 30 wt%

30
Formulation 5:
(Co)polymer: Library 2, well D9 (S/AA/4-VP = 30/17/53
                                   mol%)

Residual monomer by ^{1}H-NMR:	00/07/03 (S/AA/4-VP wt%)
Aqueous system:	500 µl buffered solution of pH = 9 (boric acid 0.05 M and potassium chloride 0.05 M)
Total solids content:	0.5 wt% solids
5 wt dye/wt total solids:	40 wt%

Formulation 6:

(Co)polymer:	Library 2, well E9 (S/AA/4-VP = 20/20/60 mol%)
10 Residual monomer by ^{1}H-NMR:	00/07/02 (S/AA/4-VP wt%)
Aqueous system:	500 µl buffered solution of pH = 9 (boric acid 0.05 M and potassium chloride 0.05 M)
Total solids content:	0.5 wt% solids
wt dye/wt total solids:	40 wt%

15

Many other formulations were made with using the entire composition space of the (co)polymer ternaries, buffering conditions from pH = 5 to 9 to unbuffered, solids content from 0.1 to 5.0 wt%, and substrate loading (wt dye/ wt of total solids) from 1 to 40 wt%.

20

Dispersion formulation process

In a preferred embodiment of the dispersion formulation process, a parallel pipettor was used to dispense 500 µl of the appropriate aqueous solution into the vials containing the dye /(co)polymer solid solutions. Mild agitation was provided
25   by repeatedly aspirating and dispensing fluid from the vials. Additional low energy agitation was provided using a small, bench-top ultrasonic mixer. In an alternative embodiment, additional agitation was delivered after heating the dispersions to 50°C for 20 to 60 minutes and using a parallel pipettor to repeatedly aspirate and dispense fluid from the vials.

30

3.   Stability testing

Samples were screened of quantity active ingredient dispersed and average particle size of dispersed particles between 12 hours and two weeks after formation of the nanodispersion. To accelerate any aging process (Ostwald ripening, crystallization, agglomeration, flocculation, chemical attack or other destabilizing processes) samples are thermally cycled. In a preferred embodiment of the aging process, samples were heated at least two times to 50°C for 1 hour and allowed to cool to ambient temperature for 4 hours. Samples were then allowed to set undisturbed for the aging process to allow unstable particles to settle to the bottom of the vials.

4.   Dispersion characteristics

Formulations 1 to 6 showed no visible precipitation following dispersion, nor after a thermal cycling and one day aging process. Spectrophotometer measurements of the samples show that samples drawn from the top and bottom of the vials show a large absorption maximum at a wave length of approximately λ = 550 nm and with less than 10% deviation between the measured absorption between samples drawn from the top and samples drawn from the bottom of the vial. In a preferred embodiment of the screen, samples were diluted to 0.05 wt% solids in the appropriate aqueous medium.

5.   Average particle sizes (by Fiber Optic DLS measurements)

Average particle sizes were determined in a fiber optic dynamic light scattering apparatus. Samples were diluted to approximately 0.005 wt% solids in the appropriate carrier aqueous solution. Particle sizes and PDI (polydispersity index) values were determined by second order cumulant analysis and are reported in terms of hydrodynamic radius (r_H). In the following tables the particle size results and PDI values from two independently dispersed systems of each formulation are shown:

Hydrodynamic radius (r$_H$)

r$_H$ [nm]	Form.[1] 1	Form. 2	Form. 3	Form. 4	Form. 5	Form. 6
Copy 1	41	44	46	41	24	20
Copy 2	30	27	_[2]	50	25	20

1) Formulation

2) no data

5   *PDI value*

PDI	Form.[1] 1	Form. 2	Form. 3	Form. 4	Form. 5	Form. 6
Copy 1	0.40	0.48	0.04	0.31	0.21	0.29
Copy 2	0.16	0.39	_[2]	0.77	0.23	0.18

1) Formulation

2) no data

Claims:

1.   A method of making an array of n nanoparticular dispersion formulations, wherein

5   said nanoparticular dispersion formulations each comprise the following
        components

        -    at least one nanodispersant,

        -    at least one application media

10   -   an active ingredient

        said method comprising the following steps

        c)   making said array of n nanoparticular dispersion formulations by

            c1)   a parallelized solid solution route, or

15   c2)   a parallelized general precipitation route, or

            c3)   a parallelized reactive precipitation route,

        d)   parallelized, rapid serial or semi-parallel characterizing of said obtained n
                nanoparticular dispersion formulations,

        wherein said active ingredient is the same in each of said n nanoparticular

20   dispersion formulations; and

        wherein n is at least 2 and wherein at least one parameter selected from the group
        consisting of components employed, concentration of the components, temperature,
        reaction time, pH-value, other useful components and solvent, if employed, is
        different in each of said nanoparticular dispersion formulations.

25

2. The method as claimed in claim 1, said method further comprising the following step
    prior to step c)

a)   a method of mak___ __ ___, of m nanodipersants by a parallel
        polymerization process of at least 2 monomers A and B,

wherein m is independent of n at least 2.

5   3. The method as claimed in claim 1, said method further comprising the following steps
        prior to step c)

    a)   a method of making an array of m nanodipersants by a parallel
            polymerization process of at least 2 monomers A and B, and

10 b)   characterizing said nanodipersants,

    wherein m is independent of n at least 2.

    4. The method of claim 1 wherein said application media is an aqueous system.

15 5. The method as claimed in claim 2 wherein said polymerization process in step a) is
        a polymerization reaction in solvent or in bulk preferably selected from the group
        consisting of radical polymerization, cationic polymerization, anionic
        polymerization, polycondensation, polyaddition, polymer analogous reactions,
        living radical polymerization, homogeneously catalyzed reactions, ring opening
20   polymerizations.

    6. The method of claim 2 wherein said method of making an array of m
        nanodipersants by a parallel polymerization process in step a) is carried out in
        solvent and comprises the steps

25

    a1)   delivering said at least 2 monomers A and B, at least one solvent and
            optionally other components appropriate for the employed polymerization
            process to each synthesis region on a substrate having k physically separate
            synthesis regions for m different nanodispersants,

a2)   subsequently or concurrently reacting said monomers and optionally other useful components to form m different nanodispersants,

a3)   optionally delivering said other components deferred to each sythesis region,

wherein at least one parameter selected from the group consisting of monomers or other components employed, concentration of said monomers or said other components, solvent employed, temperature, and reaction time, is different in each of said m nanodispersants and k is independent of m at least 2, with the proviso that $k \geq m$.

7.   The method of claim 6 wherein said physically separate synthesis regions on said substrate are wells on a microtiterplate reactor or vials of a parallel reactor.

8.   The method of claim 6 wherein said at least 2 monomers A and B, said solvent and optionally said other components appropriate for the employed polymerization process are delivered to said synthesis regions on said substrate from a pipette by automated and/or parallel delivering.

9.   The method of claim 3 wherein said nanodispersants are characterized in step b) by rapid gel permeation chromatography (GPC).

10.   The method of claim 1 wherein the parallelized solid solution route of step c1) comprises the following steps

c11)   delivering at least one of said nanodipersants and an active ingredient to each synthesis region on a substrate having n physically separate synthesis regions;

c12)   forming n solid solutions ot said at least one nanodipersant and said active ingredient,

c13)   dispensing said at least one application media into said k physically separate synthesis regions on said substrate,

wherein k is independent of n at least 2, with the proviso that k ≥ n.

11.   The method of claim 10 wherein said physically separate synthesis regions on said substrate are wells on a microtiterplate reactor or vials of a parallel reactor.

12.   The method as claimed in claim 10 wherein said solid solutions are formed by spray drying, vacuum drying or lyophilization or other drying techniques, for example by circulating warm dry air across the samples at ambient pressure.

13.   The method of claim 10 wherein said at least one nanodipersant and said active ingredient are delivered to said synthesis regions on said substrate from a pipette by automated and/or parallel delivering.

14.   The method of claim 1 wherein the parallelized general precipitation route of step c2) comprises the following step

c21)   delivering a first solution of at least one of said application media and a second solution of said active ingredient with said first and/or second solution additionally comprising said at least one nanodispersant, to each synthesis region on a substrate having k physically separate synthesis regions,

wherein the solvents of said solutions are miscible and choosen in a way that for the resulting mixture of said solvents the saturation concentration of said active ingredient will be exceeded to form n different nanoparticular dispersion formulations and wherein k is independent of n at least 2 with the proviso that k ≥ n.

15.   The method of claim 14 wherein said physically separate synthesis regions on said substrate are wells on a microtiterplate reactor or vials of a parallel reactor.

16.   The method of claim 14 wherein said at least one nanodipersant and said active ingredient are delivered to said synthesis regions on said substrate from a pipette by automated and/or parallel delivering.

17.   The method of claim 1 wherein the parallelized reactive precipitation route of step c3) comprises the following steps

        c31)   delivering a solution of one or more reactive precursor of said active ingredient and a solution of said at least one nanodispersant to each synthesis region on a substrate having k physically separate synthesis regions,

        c32)   reacting said one or more precursor to produce said active ingredient which is stabilized by said at least one nanodispersant to form n different nanoparticular dispersion formulations.

18.   The method of claim 17 wherein said physically separate synthesis regions on said substrate are wells on a microtiterplate reactor or vials of a parallel reactor.

19.   The method of claim 17 wherein said at least one nanodipersant and said active ingredient are delivered to said synthesis regions on said substrate from a pipette by automated and/or parallel delivering.

20.   The method of claim 1 wherein the parallelized characterizing of said obtained n nanoparticular dispersion formulations (step d) is an optical method selected from the group consisting of visual inspection of the nanoparticular dispersion formulations, parallel measurement of optical transmission at selected wavelength and rapid parallel or serial quasielastic light scattering.

21.   An array of at least 8 different nanoparticular dispersion formulations on a substrate
        at known locations thereon, wherein said nanoparticular dispersion formulations
        containing

        -     at least one nanodispersant,

        -     at least one application media, and

        -     an active ingredient,

        wherein said active ingredient is the same in each of said nanoparticular dispersion
        formulations.

22.   The array of claim 21 wherein said application media is an aqueous system.

23.   The array of claim 21 wherein the average particle size, reported in terms of
        hydrodynamic radius, of the dispersed particles in said dispersion formulations is
        from 10 nm to 5 μm, preferably from 10 to 500 nm and more preferably from 20 to
        50 nm.

24.   A method of making an array of m nanodipersants by a parallel polymerization
        process comprising the steps

        •   delivering at least 2 monomers A and B, optionally a solvent and optionally
              other useful components appropriate for the employed polymerization process
              to each synthesis region on a substrate having k physically separate synthesis
              regions for m different nanodispersants,

        •   subsequently or simultaneously reacting said monomers and other useful
              components to form m different nanodispersants,

wherein at least one parameter selected from the group consisting of monomers or other useful components empolyed, concentration of said monomers or said other useful components, solvent employed, temperature, reaction time is different in each of said m nanodispersants and wherein k and m are independent of of each other at least 2 with the proviso that $k \geq m$.

5

25. An array of at least 8 different nanodispersants on a substrate at known locations thereon, wherein said nanodispersants are synthesized by polymerization of at least 2 monomers A and B, wherein one of said monomers is a hydophilic monomer and the other monomer is a hydrophobic monomer.

10

26. A method of making an array of n solid solutions by a parallelized solid solution route comprising the following steps

- delivering at least one said nanodipersant and a solution of an active ingredient to each synthesis region on a substrate having k physically separate synthesis regions;

15

- forming n solid solutions of said nanodipersant and said active ingredient, wherein said active ingredient is the same in each of said n solid solutions; and

wherein k and n are independent of of each other at least 2 with the proviso that $k \geq n$.

20

27. The method as claimed in claim 26 wherein said solid solutions are formed by spray drying, vacuum drying or lyophilization or other drying techniques, for example by circulating warm dry air across the samples at ambient pressure.

25

28. An array of at least 8 different solid solutions on a substrate at known locations thereon, wherein said solid solutions comprising the following components

- at least one nanodispersant, and

54

- an active ingredient;

wherein said active ingredient is the same in each of said solid solutions.

5   29.   A process for the production of 100 to 100,000, preferably more than 1000 to 100,000, more preferably 10,000 to 100,000 nanoparticular dispersion formulations a day, each comprising the following components

- at least one nanodispersant,

10 - at least one application media

- an active ingredient;

wherein said active ingredient is the same in each of said nanoparticular dispersion formulations.

15

30.   A process for the production of 100 to 100,000, preferably more than 1000 to 100,000, more preferably 10,000 to 100,000 nanodispersants a day, by a parallel polymerization process of at least 2 monomers A and B.

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ARTICLES

Water-Compatible Molecularly Imprinted Polymers Obtained via High-Throughput Synthesis and Experimental Design

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Abstract: A technique allowing high-throughput synthesis and evaluation of molecularly imprinted polymer sorbents at a reduced scale (mini-MIPs) was developed and used for the optimization of MIPs for use in pure aqueous environments. The technique incorporated a 4-port liquid-handling robot for the rapid dispensing of monomers, templates, solvents and initiator into the reaction vessels of a 96-well plate. A library of 80 polymers, each ca. 50 mg, could thus be prepared in 24 h. The MIP rebinding capacity and selectivity could be rapidly assessed in the batch mode by quantifying nonbound fractions in parallel using a UV monochromator plate reader. This allowed a complete evaluation of the binding characteristics of an 80 polymer library in approximately 1 week. With the objective of optimizing a polymer imprinted with the local anaesthetic Bupivacaine for use in pure aqueous systems, a polymer library was prepared by varying the original poly(MAA-co-EDMA) MIP composition. The variable factors were the added amount of the hydrophilic comonomer, 2-hydroxyethyl methacrylate (HEMA), the cross-linking ratio, and the porogen. This optimization resulted in polymers showing high imprinting factors (IF = K_{MIP}/K_{NIP}) in water as a result, mainly, of reduced binding to the nonimprinted polymer. Normal scale batches of these materials showed strong retention of the template and low nonspecific binding when assessed as chromatographic stationary phases using pure phosphate buffer, pH 7.4, as mobile phase, by equilibrium batch rebinding experiments and as sorbents for extractions of the analyte from blood plasma samples.

Introduction

Molecular imprinting technology is attracting widespread attention due to its potential to deliver robust molecular recognition elements targeted toward essentially any guest present in any environment (e.g. drug enantiomers, hormones, toxins, pesticides, peptides, proteins, and nucleic acids in matrixes ranging from pure organic solvents to biological fluids).[1-3] The previously developed imprinting protocols can be successfully used to produce molecularly imprinted polymers (MIPs) for recognition of a large range of guest molecules predominantly in organic solvent-based media. Although some MIPs synthesized by the use of specifically designed monomer—solvent combinations[4-7] or by the conventional imprinting protocol based on poly(MAA-co-EDMA)[8,9] exhibit recognition properties under aqueous conditions, current technology often fails to generate MIPs for use in pure aqueous environments.

This is often due to nonspecific hydrophobically driven binding,[9,10] the extent of which depends on the hydrophobicity of the template and the exposed surface of the material. Supressing the nonspecific binding may result in MIPs being closer antibody mimics, which hence can be implemented in separations or chemical sensors in aqueous environments such as biological fluids and environmental waters.

Due to the many parameters influencing the materials' properties at different length scales, as well as the absence of a clear understanding of how these parameters interplay, there are presently no well developed rules to follow for the design of materials exhibiting the desired recognition properties. Thus,

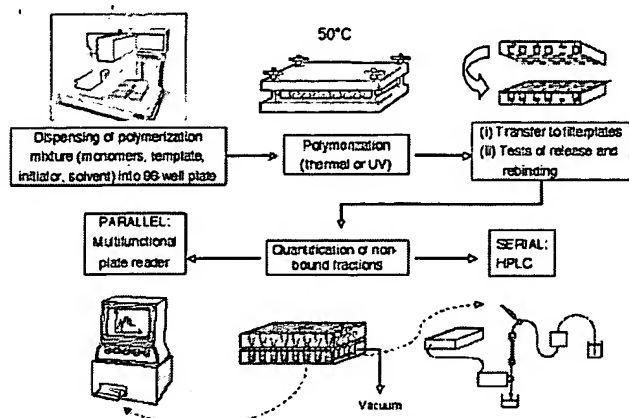† Johannes Gutenberg Universität Mainz.
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Figure 1. Procedure for high-throughput synthesis and evaluation of large groups of polymers.

Figure 2. Scheme showing the imprinting of Bupivacaine (BV) in poly-(MAA-*co*-EDMA) and factors considered in the synthesis optimization.

combinatorial synthesis approaches, allowing the main factors to be rapidly screened, have offered valuable tools in the development of new MIPs.

We and others recently introduced an in situ synthesis and evaluation technique for MIPs, resulting in libraries of mini-MIPs at the bottom of HPLC-autosampling vials.[11-13] The recognition properties of the polymers could be assessed in situ by HPLC quantification of the nonbound fraction of the template at equilibrium. These techniques were time-consuming due to the slow removal of template and the need for serial analysis of the supernatant solutions. In this report we have cirumvented these problems by the use of filter plates for rapid template removal and a multifunctional plate reader for a parallel analysis of the supernatant fractions (Figure 1).

A complete 96-well plate library can thus be synthesized and evaluated in approximately 1 week, which should be compared with the 3−4× longer time required using the original mini-MIP system. This high-throughput synthesis and screening (HTS) system allows the combinatorial synthesis of large libraries of MIPs with rapid replacement of the liquid phase in the release and rebinding experiments. By using the techniques of experimental design and multivariate analysis,[14] the system constitutes a powerful tool for the rapid optimization of MIPs to attain the desired performance.

Here we have used the HTS system for the optimization of MIPs for use in solid-phase extraction (SPE)[15] targeted toward the local anaesthetic Bupivacaine (Figure 2).

Under aqueous conditions, the hydrophobic surface of these polymers leads to substantial nonspecific binding of the template bupivacaine, as well as nonspecific retention of nonrelated, nonpolar structures.[9,16] In addition, biological sample components, such as proteins and lipids, are strongly adsorbed to the polymer surface. Both processes lead to gradual deterioration of the analytical performance of the extraction and chromatographic columns. In some cases these can be restored by suitable washing schemes, but often, however, the only resort is a
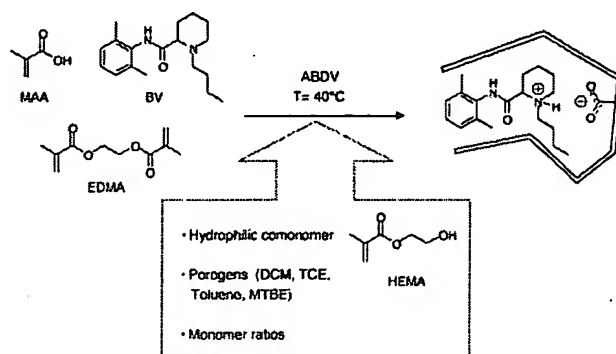
frequent change to fresh columns or, alternatively, the use of additional sample pretreatment procedures to remove harmful matrix components.

The ultimate aim was therefore to obtain imprinted sorbents capable of selective extraction of the analyte from pure aqueous buffer with minimum nonspecific binding of the drug as well as other matrix components. This would obviate the need for organic solvent based washing steps. The starting point for the optimization was the well-characterized MIP consisting of poly(MAA-*co*-EDMA) imprinted with Bupivacaine (Figure 2).[16] This polymer was chosen as the reference for subsequent comparisons. The library was constructed by slightly modifying the procedure used to make the reference polymer. The modifications comprised the following: (1) the use of the hydrophilic comonomer 2-hydroxyethyl methacrylate (HEMA), known to impart water compatibility in a number of unrelated systems;[17-20] (2) the use of four porogens (DCM, TCE, toluene, and MTBE), chosen considering health risks, volatility, hydrogen bond capacity, and polarity; (3) the relative ratios of (A) HEMA/MAA and (B) (HEMA + MAA)/EDMA. For each porogen, the latter factors were optimized by a 2^2 factorial design experiment including one center point. The chosen response factors were the partition coefficients of the template on the MIP (K_{MIP}) and on the NIP (K_{NIP}) and the imprinting factor, defined as IF = K_{MIP}/K_{NIP}, the latter reflecting the affinity and concentration of imprinted sites. The best performing polymers were upscaled for assessment as stationary phases by liquid chromatography, by competitive rebinding experiments in aqueous buffers, and as sorbents for extractions of Bupivacaine from blood plasma samples.

Experimental Section

Chemicals. The hydrochloride salts of Bupivacaine (BV), Ropivacaine (RV), and Mepivacaine (MV) were provided by Astra-Zeneca R&D Södertälje (S-15185 Södertälje, Sweden).

The template (BV) was transformed into the free base as follows: BV HCl (100 mg) was dissolved in water (15 mL). After the pH was adjusted to 11 with Na_2CO_3, Bupivacaine (BV) was extracted into dichloromethane (DCM, 3 × 10 mL). After washing of the organic phase with water (10 mL), it was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure, yielding the free base quantitatively.

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Table 1. Stock Solutions and Dispensing Scheme for the Preparation of the MiniMIP Library

F1 [(HEMA + MAA)/ EDMA]	F2 (HEMA/ MAA)	MAA (mg)	HEMA (mg)	porogen (µL)	stock soln/mini-MIP (µL)	EDMA/mini-MIP (µL)
1/5	0/1	43	0	400	34	48
1/5	2/1	14	43	400	35	48
1/1	0/1	129	0	400	43	29
1/1	2/1	43	130	400	46	29
1/2	1/1	43	65	400	40	38

Methacrylic acid (MAA), 2-hydroxyethyl methacrylate (HEMA), and ethylene glycol dimethacrylate (EDMA) were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) and purified prior to use as follows: MAA and HEMA were distilled under reduced pressure; EDMA was washed consecutively with 10% NaOH, water, and brine and then dried over $MgSO_4$, filtered, and distilled under reduced pressure. The initiator azo-N,N'-bis(divaleronitrile) (ABDV) was purchased from Wako Chemicals and used without further purification.

Anhydrous dichloromethane (DCM), anhydrous toluene, methyl tert-butyl ether (MTBE), acetonitrile (ACN) for HPLC, methanol (MeOH) for HPLC, water for HPLC, Tween 20, and acetic acid (AcOH) biochemical grade were purchased from Acros (Geel, Belgium). Ethanol came from Kemetyl (Haninge, Sweden). 1,1,1-Trichloroethane (TCE), citric acid, and the buffer salts, Na_2HPO_4, sodium citrate, and CH_3COONH_4, were purchased from Merck (Darmstadt, Germany). All porogens were kept under an argon atmosphere over molecular sieves and were used without further purification. Human albumin (lyophilized) from human serum was purchased from Serva-Heidelberg and tritium-labeled Bupivacaine was obtained from Moravek Biochemicals (Brea, CA). Scintillation cocktail fluid was purchased from Wallac (Turkku, Finland). The water used in the HPLC study was obtained from a Milli-Q unit equipped with a Quantum VX ultrapure V-Ionex cartridge from Millipore.

Apparatus. The 96-well PTFE microtiter plate and PTFE coated closures were obtained from Radleys (Shire Hill, Saffron Walden, Essex, U.K.). The chemically resistant 96 filter- and microtiter plates were a gift from Whatman Polyfiltronics (Maidstone, Kent, U.K.).

The 96 microtiter glass plates and PTFE-coated silicon Septa were obtained from Zinsser Analytic (Frankfurt, Germany).

Quartz-glass microtiter plates were obtained from Hellma Worldwide (Müllheim, Germany).

All chromatographic evaluations were performed using a Hewlett-Packard instrument (HP 1050) equipped with a quaternary pump, an autosampler, a diode array detector, and an HP workstation. The parallel UV measurements were performed using a multifunctional plate reader SAFIRE, from Tecan Deutschland GmbH (Crailsheim, Germany). For pipetting of the polymer solutions a 4-port liquid sample handler LISSY from Zinsser Analytic (Frankfurt, Germany), equipped with Zinsser WinLissy software, was used.

The pipetting of washing solutions and the preparation of monomer solutions was performed with Eppendorf Research Pro 8-manifold pipets (Eppendorf AG, Hamburg, Germany).

The nitrogen sorption study was performed on a Quantachrome Nova 2000 (Quantachrome Corp., Boynton Beach, FL).

The equipment used for the equilibrium rebinding and competitive rebinding experiments included an Eppendorff centrifuge from Hettich (Tuttlingen, Germany) and a WinSpectral 1414 scintillation counter from Wallac (Turku, Finland).

Mini-MIP Library. (a) Synthesis. For each of the four porogens (DCM, TCE, toluene, MTBE), two initiator solutions (with and without template) were prepared by mixing ABDV (24 mg) with 640 µL of the porogen. For the MIP series, BV (19.4 mg) was added as template. Five different stock solutions of the functional monomers were prepared per porogen as specified in Table 1. Prior to preparation of the solutions

the porogens, EDMA, MAA, and HEMA were purged with argon for 2 min.

The initiator solution (40 µL with or without template) was then dispensed into the 96-well PTFE microtiter plate, followed by addition of the functional monomer stock solutions and the cross-linker EDMA (see Table 1).

Prior to the polymerization, the microtiter plate was sealed with a PTFE-coated silicon septum. Each pipetting step was accompanied by degassing with argon for 5 s.

The microtiter plate was sealed with Viton rings and a PTFE cover plate and then heated in oven for 24 h at 50 °C.

(b) Template Release, Extraction, and Rebinding Experiments. After polymerization the polymers were transferred to a 96-well filter plate. The template was extracted by successive washing steps with 600 µL of MeOH/AcOH/H_2O (60/30/10, v/v/v) until the template could no longer be detected in the washing solution. This was followed by a conditioning step with methanol. Prior to the rebinding experiments, the library was subjected to a final wash with the same solvent as used in the rebinding step.

The rebinding experiments were then performed by adding 600 µL of a solution of BV (1 mM) in ACN (HPLC grade) or BV as its HCl salt (1 mM) in NaH_2PO_4/Na_2HPO_4 buffer solution (HPLC grade) (25 mM, pH 7.4) respectively to each well of the microtiter plate.

The concentration of free BV was determined after 16 h by direct absorbance reading using a multifunctional plate reader or by sequential HPLC analysis. In both cases the following procedure was followed: After addition of the incubation solutions the filter plate was sealed on top and bottom with PTFE-coated silicon closures with the aid of a press. After the incubation of the polymers the closures were removed and the solutions were sucked through undervacuum into a microtiter plate from which samples were taken for the subsequent measurements. For the evaluation using the plate reader, 200 µL samples from each well were transferred into a 96-well quartz plate and measured at 230 nm. For the HPLC evaluation 10 µL samples were injected and analyzed using a C18 reversed-phase column (EU Material UMMA 049S, 5 µm, 150 × 4 mm), with the mobile phase ACN/10 mM ammonium acetate buffer (pH 4.5) (70/30, v/v), UV detection at 230 nm, and the BV/BV HCl rebinding solution as external standard. After each rebinding experiment the polymers were reconditioned by first washing with MeOH/AcOH/H_2O (60/30/10, v/v/v) until template could no longer be detected, followed by a wash with the rebinding solvent.

Normal Scale Batches. Upscaled versions of the polymer pairs (imprinted and nonimprinted) 9 and 14, as well as the reference polymer pair 11, were prepared as follows. MAA (340 µL, 4 mmol), HEMA (MIP/NIP 9 and 14, 970 µL, 8 mmol; MIP/NIP 11, no HEMA), EDMA (MIP/NIP 9 and 14, 2.5 mL, 12 mmol; MIP/NIP 11, 3.8 mL, 20 mmol), ABDV (MIP/NIP 9 and 14, 60 mg, 0.24 mmol; MIP/NIP 11, 110 mg, 0.44 mmol), and BV (all MIPs: 97 mg, 0.33 mmol) were dissolved in the porogen (5.6 mL of TCE (MIP/NIP 9); 5.6 mL of toluene (MIP/NIP 14); 5.2 mL of toluene (MIP/NIP 11)) and then transferred to glass polymerization tubes (14 mm i.d.). Each solution was then degassed with nitrogen for 5 min, and the tubes were sealed and heated at 40 °C for 24 h. After the polymerization, the tubes were smashed and the polymer monolith was coarsely ground and then extracted with MeOH for 24 h in a Soxhlet apparatus. Thereafter the particles were further crushed first with a mortar and pestle and then in a ball-mill, sieved under water, and dried at 40 °C.

Chromatographic Evaluations. The particles (25−50 µm size fraction) were slurried in MeOH/water (80/20, v/v) and packed into HPLC columns (30 mm × 4 mm) at a maximum pressure of 200 bar using a compressed gas-driven slurry packer and MeOH/water (80/20, v/v) as pushing solvent. Thereafter the polymers were tested by comparing the retention factors (k) for BV injected as 10 µL of 10 mM solutions of BV dissolved in ACN or BV HCl dissolved in water on the different polymers in different mobile phases. The retention factors were calculated from the estimated retention times (t) of the

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peak maxima and the elution time of the void marker (t_0) acetone or MeOH as $k = (t - t_0)/t_0$. The UV detection wavelength was 230 nm and the flow rate 1.0 mL/min.

Swelling Tests. The polymer volume swelling was estimated using volume-calibrated NMR tubes filled with 0.5 mL of well-packed polymer particles (25−50 μm size fraction). After addition of solvent (ACN or water), the tubes were allowed to stand at room temperature until no further change of the swollen bed volume was observed. The swelling was determined as the ratio of the swollen bed volume to the dry bed volume.

Equilibrium Rebinding and Competitive Rebinding Experiments. Prior to both the equilibrium rebinding and the competitive rebinding experiments, a 20 mg/ mL suspension of polymer particles (25−50 μm size fraction) was prepared and left for 24 h. Before the equilibrium rebinding experiments, 1 mL of incubation solutions was prepared in Eppendorf tubes. These contained 400 μL of citrate buffer (125 mM, pH 5), ethanol (5%), Tween 20 (0.05%), and radiolabeled Bupivacaine (50 μL, ca. 1.2 ng) (30 000−50 000 dpm) as well as 0, 0.25, 0.5, 1.0, 2.5, 5, or 10 mg of polymer added from the above stock suspension. The final volume was adjusted to 1 mL with water. The Eppendorf tubes were placed on a rocking bed for 16 h to allow equilibration. After 16 h the tubes were removed and centrifuged at 18 000 rpm for 5 min and 0.5 mL of supernatant was removed. To the supernatant, 5 mL of scintillation fluid was added, and scintillation counting of the free radiolabeled Bupivacaine was performed.

For the competitive rebinding experiments, 1 mL of incubation solutions was prepared in Eppendorf vials. These contained 400 μL of citrate buffer (125 mM, pH 5), ethanol (5%), Tween 20 (0.05%), and radiolabeled Bupivacaine (50 μL, ca. 1.2 ng) (30 000−50 000 dpm). The amount of polymer added to the Eppendorf tubes was equivalent to the PC_{50} values calculated from the equilibrium rebinding experiments. These values were 1.3 mg/mL for MIP 11, 5.0 mg/mL for NIP 11, 4.3 mg/mL for MIP 9. and 7.2 mg/mL for the MIP 14. Finally, a competing analyte, Bupivacaine, Ropivacaine, or Mepivacaine, was added at varying concentrations between 1 and 330 000 nM. As previously described for the equilibrium rebinding experiments, the Eppendorf tubes were placed on a rocking bed for 16 h and centrifuged and the supernatant was counted after addition of 5 mL of scintillation cocktail.

Protein Binding. Adsorption of human serum albumin on polymers 9, 11, and 14 was tested using the same columns as used in the chromatographic evaluations (vide supra). Before use, the columns were preconditioned by successive washings with water, HCl (0.07 M), water, MeOH/water (50/50), water, and finally 25 mM phosphate buffer (pH 7.4). The adsorption test was performed in 25 mM phosphate buffer at pH 7.4 at a flow rate of 1 mL/min with a UV detector recording at a wavelength of 290 nm. A human serum albumin solution (50 mg/mL) made up in the mobile phase buffer was injected (100 μL) four times consecutively. The amount of adsorbed protein after each injection was calculated from the area of the breakthrough peak in relation to the area of the peak obtained after injection of the protein solution in absence of a column.

Solid-Phase Extraction. Two standard polypropylene SPE cartridges containing each 24 mg of polymer were prepared for each polymer. Prior to use, they were conditioned by washing with 1 mL of methanol followed by 1 mL of water. The plasma sample was prepared as follows. To a plasma sample (400 μL) was added nonlabeled Bupivacaine (1000 nM) and 100 000 DPM (approximately 10 nM) of labeled Bupivacaine, ethylcaine (8 μM) in water (100 μL), and citrate buffer (0.4 M, pH 5, containing 0.1% Tween 20) (500 μL). This sample was then applied at the top of the column. This was followed by a wash step with 2 × 1 mL water, one wash step with 0.5 mL of acetonitrile and two elution steps with 2 × 1 mL of 92% acetonitrile, 6% water, and 2% TEA. To 0.1 mL of each fraction was added 5 mL of scintillation fluid followed by scintillation counting.

Results and Discussion

Choice of Factors and Experimental Design. The starting point for the optimization was the previously reported MIP of the poly(MAA-*co*-EDMA)-type targeted toward Bupivacaine (Figure 2). As with the majority of MIPs described in the literature, this material is of the macroreticulate type prepared by free radical polymerization in the presence of high levels of cross-linking monomers and a porogenic solvent.[21] This gives rise to amorphous materials containing nanometer-sized binding sites in addition to larger sized pores. In this one-step approach, the successful imprinting of a particular template depends on a simultaneous fulfillment of several criteria. First, molecular binding sites for the template and the target molecule(s) need to be generated at or near the pore walls. Second, the surface of the material must be compatible with the medium of application. Most MIPs prepared by the self-assembly approach contain a methacrylate- or styrene-based polymer backbone which imparts a respectively slight or pronounced hydrophobic character to the material.[22] While these materials commonly exhibit pronounced recognition in low dielectric strength media, a hydrophobically driven nonspecific adsorption is observed when they are used in water. On the other hand, the surface of hydrophilic materials (e.g. poly(acrylamides), poly(HEMA)) are wetted by water and exhibit low nonspecific adsorption in such media.

Typically, the generation of stable high affinity imprinted sites requires the following:[21]

(i) one or more functional monomers capable of forming stable complexes with the template molecule during polymerization; (ii) a high nominal cross-linking level as lower levels (<50%) are insufficient for preserving the templated sites for longer periods of time; (iii) the use of an aprotic apolar solvent as porogen as this favors the electrostatic interactions most commonly utilized between the functional monomers and the template.

These requirements are to some extent contradictory to the approaches available to incorporate hydrophilic surface properties:

(1) Polar porogens[22] can be used. These solvate the polar functional groups of the monomers leaving them exposed at the pore walls after porogen removal. This in turn leads to reduction of hydrophobic nonspecific binding.

(2) Hydrophilic comonomers (e.g. HEMA, acrylamide) or cross-linkers (e.g. pentaerythritoltriacrylate, methylenebis(acrylamide)) can be used in the imprinting step.[5−7,17−20] Depending on the polarity of the porogen these will be more or less exposed at the pore walls of the materials.

(3) Postgrafting of hydrophilic chains can be employed.[23]

In view of the recent reports on imprinted HEMA-based hydrogels,[17] we decided to investigate approach 2 by addition of HEMA under conventional imprinting conditions satisfying the criteria for binding site stabilization. Thus, terpolymers of the type poly(MAA-HEMA-EDMA) were prepared in the presence of four different aprotic porogens. Due to the complex-

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Table 2. Monomer Compositions Used To Prepare the Polymer Library and Results from the Rebinding Tests of Bupivacaine-Imprinted and Nonimprinted Polymers in Acetonitrile[a]

MIP/NIP	porogen	F1 [(HEM A + MAA)/EDMA]	F2 (HEMA/MAA)	HPLC K_{MIP} (mL/g)	HPLC K_{NIP} (mL/g)	HPLC IF	reader K_{MIP} (mL/g)	reader K_{NIP} (mL/g)	reader IF
1	DCM	1/5	0/1	49.8 ± 3.2	21.3 ± 1.9	2.3	50.7 ± 3.3	22.0 ± 1.3	2.3
2		1/5	2/1	10.5 ± 0.6	6.1 ± 0.1	1.7	11.2 ± 0.5	6.3 ± 0.0	1.8
3		1/1	0/1	281 ± 21	161 ± 0.8	1.7	272 ± 19	143 ± 1.5	1.9
4		1/1	2/1	37.2 ± 0.3	26.4 ± 1.1	1.4	49.7 ± 5.7	27.1 ± 0.9	1.8
5		1/2	1/1	61.1 ± 2.5	26.7 ± 0.3	2.3	58.9 ± 2.4	26.3 ± 0.8	2.2
6	TCE	1/5	0/1	58.0	13.3 ± 0.0	4.4	61.4	15.3 ± 0.5	4.0
7		1/5	2/1	7.5 ± 0.0	3.6 ± 0.1	2.1	11.6 ± 1.6	3.6 ± 0.2	3.3
8		1/1	0/1	83.5	12.3 ± 0.7	6.8	31.1	12.8 ± 0.9	2.4
9		1/1	2/1	14.7 ± 0.3	0.8 ± 0.0	20	15.3 ± 0.2	0.5 ± 0.1	33
10		1/2	1/1	49.6 ± 0.3	5.9 ± 0.0	8.4	40.1 ± 0.7	6.0 ± 0.2	6.6
11	toluene	1/5	0/1	59.8 ± 0.5	14.6 ± 0.5	4.1	60.1 ± 0.0	13.3 ± 0.2	4.5
12		1/5	2/1	7.6 ± 0.1	4.0 ± 0.3	1.9	8.5 ± 0.0	3.8 ± 0.1	2.3
13		1/1	0/1	34.3 ± 0.4	9.1 ± 0.2	3.8	33.0 ± 37	9.6 ± 0.2	3.4
14		1/1	2/1	4.9 ± 0.3	0.2 ± 0.0	21	2.3 ± 0.6	0.2 ± 0.0	9.8
15		1/2	1/1	31.1 ± 0.5	5.9 ± 0.1	5.2	31.1 ± 1.2	6.0 ± 0.0	5.2
16	MTBE	1/5	0/1	49.6 ± 0.3	17.1 ± 0.3	2.9	48.8 ± 0.0	18.3 ± 0.2	2.7
17		1/5	2/1	9.1	5.1 ± 1.0	1.8	9.4	5.2 ± 0.6	1.8
18		1/1	0/1	204 ± 40	203	1.0	173 ± 29	170	1.0
19		1/1	2/1	23.3 ± 0.1	14.8	1.6	21.9 ± 0.1	14.4	1.5
20		1/2	1/1	28.5 ± 1.1	18.4 ± 0.0	1.6	33.1 ± 3.3	17.6 ± 0.2	1.9

[a] The polymer library was prepared as described in the Experimental Section by addition of a total of 300 μmol of monomer, 4 μmol of BV, and 120 μL of porogen containing the initiator ABDV (1.5 mg) per well. The molar ratios of functional monomers to EDMA and HEMA to MAA are given as F1 and F2, respectively. After degassing and sealing, the plates were left at 50 °C for 24 h. After exhaustive extraction of the template, the rebinding test was performed by incubating each polymer with a 1 mM solution of BV in acetonitrile for 16 h. Quantification of the free BV was performed by HPLC or reader analysis of the supernatant fractions. From these values and the weight of each polymer, the partition coefficients (K) and the imprinting factor IF ($=K_{MIP}/K_{NIP}$) were calculated. Errors are given as the spread between the two replicas. No error limits are given for the members lacking replicas.

ity of the imprinting process (vide supra), the polymer composition was optimized by following the theory of experimental design. The continuous factors chosen were the molar ratio of the functional monomers to the cross-linking monomer (F1: (HEMA + MAA)/EDMA) and the molar ratio of the functional monomers (F2: HEMA/MAA). Finally, the type of porogen was chosen as a discontinuous factor (F3: DCM, toluene, TCE, MTBE). The experiment was performed according to a 2^2 full factorial design with one center point and the limits of F1 being 1/5 and 1/1, whereas F2 was varied within the interval 0/1 and 2/1. These intervals were chosen taking the above criteria for the stabilization of the monomer–template complexes and the imprinted sites into consideration. It should be noted that the lower values of F1 and F2 correspond to the conditions used to prepare the reference MIP that was previously extensively investigated as a solid-phase extraction sorbent and in competitive assays.[16] The choice of porogens was guided by previous reports, as well as by their chemical and physical properties. DCM and toluene are the most commonly employed of the poorly polar porogens and have been used to generate good binding sites for a large number of low molecular templates. 1,1,1-Trichloroethane (TCE) is similar to DCM in terms of polarity (ϵ_{DCM} = 8.93, ϵ_{TCE} = 7.24) but exhibits attractive properties in terms of its lower toxicity and higher boiling point (bp$_{DCM}$ = 40 °C; bp$_{TCE}$ = 75 °C), the latter being of particular importance when polymerizing thermally in non-pressure-proof reaction chambers. Finally, MTBE, in combination with MMA/EDMA (1/4), has been shown to generate high surface area materials comparable to or grater than those prepared using toluene, acetonitrile, or chlorobenzene.[24]

Mini-MIP Library for Bupivacaine Recognition. The composition of the polymerization mixtures of each member

of the library is seen in Tables 1 and 2. These were prepared by the use of a pipetting robot, by pipetting degassed stock solutions of the monomers, initiator, template, and porogen to the wells of a PTFE 96-well microtiter plate covered with a silicone rubber sealing mat. Each pipetting step was accompanied by 5 s degassing with argon. An 80-polymer library was prepared consisting of 20 different monomer compositions, one replica/member, and an equal number of nonimprinted control polymers. The polymerization was performed by heating the plate, sealed tightly with a PTFE lid fixed in place by the aid of a press, for 24 h in an oven. After polymerization, the wells were visually inspected for the presence of liquid, unreacted monomer, and solid polymer. For five of the members (see Table 2) no polymer was obtained, presumably due to clogging of the needle due to septum disintegration. After drying of the polymers, they were weighed and transferred mechanically to deep-well filter plates. The weights of the polymers, measured after the first rebinding experiment, were found to be between 40 and 55 mg, indicating a high conversion of the monomers.

To remove the template, the polymer library was then subjected to an exhaustive extraction by repeated additions of MeOH/AcOH/H$_2$O (60/30/10, v/v/v) whereafter the polymers were conditioned by incubating them with MeOH followed by MeCN/H$_2$O (70/30 (v/v)). After drying, the ability of the polymers to bind the template was assessed by adding 500 μL of a Bupivacaine solution (1 mM) in MeCN to each well, followed by incubation for 16 h.

The nonbound fractions were then quantified in parallel using a multifunction plate reader or in series by HPLC (triplicate injections). In the former method, the supernatants were directly vacuum transferred to a receiver plate followed by transfer to a 96-well quartz plate. The supernatant concentrations could then be directly determined from the absorbance of each well at 230

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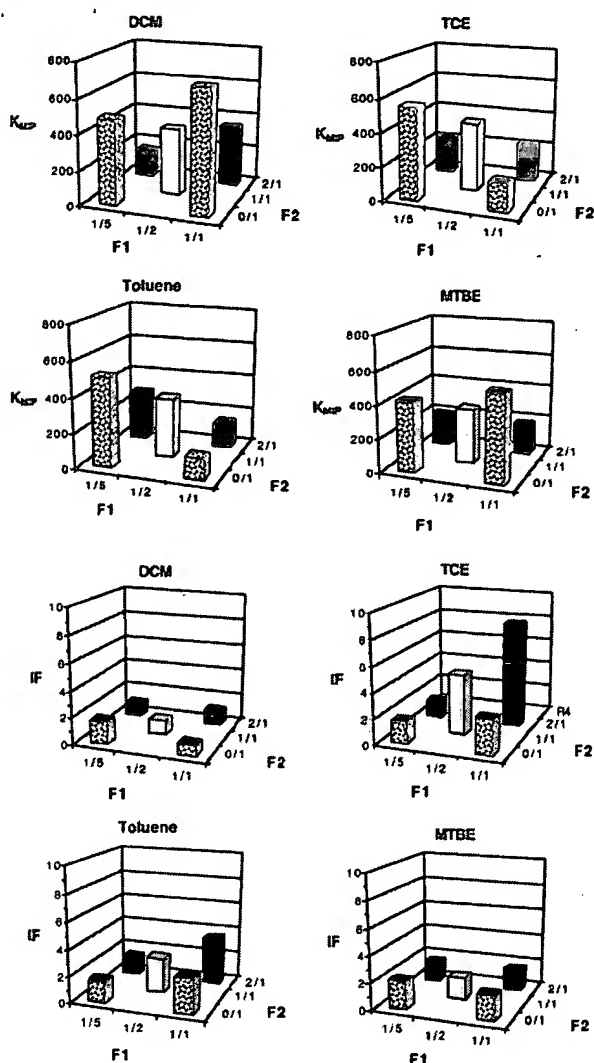
Figure 3. 3D representation of the results from the rebinding of BV–HCl in NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer solution (HPLC grade) (25 mM, pH 7.4) to the polymer library showing in (A) the partition coefficients for the imprinted polymers (K_{MIP}) and in (B) the corresponding imprinting factors (IF) calculated from the supernatant free concentrations of BV (1 mM) after binding to the polymer library. Conditions are otherwise as described in Table 2.

Table 3. Composition of Monomer Mixtures Used To Prepare the Upscaled Polymers, Selected from the Mini-MIP Library, and Structural Data for the Polymers Obtained from Nitrogen Sorption Measurements[a]

polymer	porogen	F1 [(HEMA + MAA)/EDMA]	F2 (HEMA/MAA)	S^b (m^2/g)	$V_p{}^b$ (mL/g)
MIP 9	TCE	1/1	2/1	95	0.21
NIP 9	TCE	1/1	2/1	35	0.080
MIP 14	toluene	1/1	2/1	49	0.088
NIP 14	toluene	1/1	2/1	15	0.023
MIP 11	toluene	1/5	0/1	297	0.62
NIP 11	toluene	1/5	0/1	308	0.68

[a] The polymers were prepared and characterized as described in the Experimental Section. [b] Results from nitrogen sorption isotherms calculated using the BJH method applied to the desorption branch of the isotherm. S = cumulative surface area, and V_p = the total volume of pores with diameter less than 50 nm.

functional monomer, methacrylic acid, in the preparations. Otherwise, the group of polymers prepared using TCE as porogen showed trends similar to the one prepared using toluene, with a decrease in K with increasing (HEMA + MAA)/EDMA (F1), whereas the opposite was observed for the other two groups prepared using DCM and MTBE as porogens. This groupwise behavior is also reflected in the imprinting factors (IF). Here the DCM and MTBE groups all exhibit IFs between 2 and 3 whereas the TCE and toluene groups, as a whole, showed higher IFs, with the highest values (IF $\approx$ 20) observed for the polymers 9 and 14 prepared using high ratios of HEMA/MAA and (HEMA + MAA)/EDMA, F2 and F1. This is due to a strong decrease in the binding to the corresponding non-imprinted polymers.[26]

Encouraged by these results, we repeated the rebinding experiments in pure aqueous buffer. The rebinding results are best viewed in 3D diagrams (Figure 3) with F1 and F2 on the x and z axis and the response factors (K and IF) on the y axis. As can be seen in Figure 3, the results were qualitatively similar to the results in Table 2 with the highest IF again seen for the polymer prepared using TCE (MIP 9) followed by toluene (MIP 14) as porogens and the highest values of F1 and F2. This indicates that imprinted polymers exhibiting dramatically reduced nonspecific binding in pure aqueous buffers can be prepared from specific monomer and porogen compositions. To further investigate the most promising members of the library polymers 9 and 14 were prepared in larger scale together with polymers 11 used as a reference, prepared similarly to the previously investigated MIP (Table 3).

Characterization of Upscaled Batches. Polymerization and workup were carried out by following a well-established procedure.[27] Particles of the 25–50 μm size fraction were isolated and packed in stainless steel columns, and chromatographic tests were thereafter performed using first acetonitrile and then potassium phosphate buffer, pH 7.4 as mobile phases. Figure 4 shows the elution profiles obtained from 100 nmol injections of BV (in MeCN) or BV–HCl (in buffer) on the imprinted and nonimprinted polymers 14 and 11.

In pure acetonitrile the reference MIP 11 strongly retains the solute with a retention factor (k) of 11, whereas retention on

nm. Table 2 shows the average partition coefficients and imprinting factors from the two replicas using both quantification techniques.

The small difference between the values of the replicas indicates that the polymers can be reproducibly prepared in the well plate format.[25] Also gratifying is the agreement between the results obtained using the two quantification techniques. This implies that reliable quantification of the nonbound fractions of a 96-well plate library can be obtained using parallel reading in a fraction (1–2 min) of the time required using serial HPLC analysis (ca. 45 h). The above results appear particularly promising in view of the weak chromophore of the template.

The partition coefficients (K) decreased with an increasing HEMA/MAA ratio (F2). This was common for the four groups of polymers prepared using the different porogens and is likely due to a lower concentration of the more strongly interacting

(25) A repetition of the rebinding experiment after wash and conditioning of the materials gave similar results.

(26) As remarked by one reviewer, the groupwise behavior correlates with the volatility of the porogens. Thus, the poorer performance of the DCM and MTBE materials could be ascribed to partial evaporation of the porogen during polymerization. However, ligand binding results for normal scale batches corresponding to polymers 4 and 10 agreed with those of the well plate.

(27) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31.
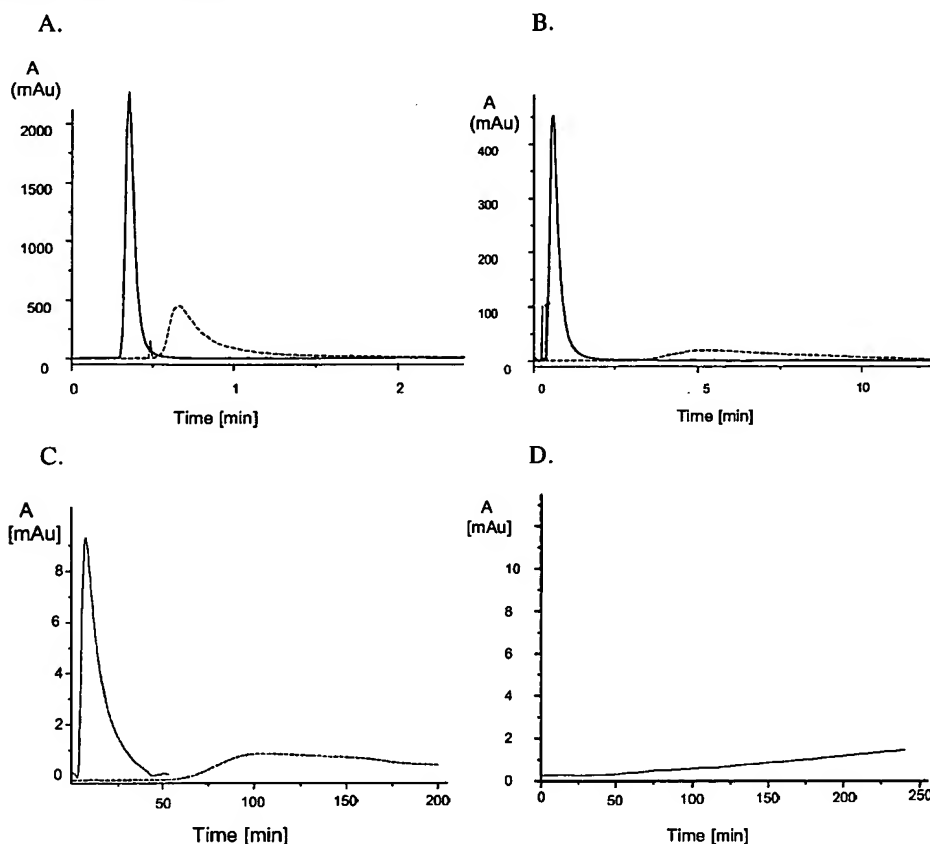
Figure 4. Comparison of the elution profiles of BV (100 nmol) obtained using polymers 14 (A, C) and polymers 11 (B, D) (MIP, dashed lines; NIP, solid lines) in acetonitrile (A, B) or NaH_2PO_4/Na_2HPO_4 buffer solution (HPLC grade) (25 mM, pH 7.4) (C, D).

the nonimprinted polymer is weak ($k = 1.25$) (Figure 4B). This contrasts with the elution profiles on polymers 14, where the solute elutes below 1 min on both the MIP ($k = 0.37$) and the NIP ($k = 0.017$) (Figure 4A).[28] Despite this apparently poor performance this polymer exhibited high imprinting factor in pure aqueous buffer (Figure 4C). Thus, the solute elutes with a peak maximum at ca. 10 min on the NIP while the peak maximum on the MIP, despite the characteristically broad peak, can be estimated at ca.100 min.[28] As seen in Figure 4D, the solute is strongly retained on the reference MIP and NIP under these conditions. These observations confirm the results obtained from the polymer library investigation and indicate that the optimized polymers exhibit considerably lower nonspecific binding in pure aqueous media compared to the reference polymer.

The selectivity of the polymers was subsequently investigated by ligand binding experiments[29] in the batch mode using radiolabeled Bupivacaine. To relate the data to previous studies using the reference polymer 11,[9] an optimized buffer composition consisting of citrate buffer (pH 5) containing ethanol (5%) and detergent (Tween 20, 0.05%) was used. The adsorption of [³H]-labeled BV was first studied as a function of the concentration of added polymer (Figure 5). The isotherms essentially confirm the results obtained using the polymers as chromatographic stationary phases.

(28) The retention factors obtained in acetonitrile using MIP and NIP 9 were 0.66 and 0.054, respectively. In pure aqueous buffer BV eluted at 32 min on NIP 9 and after more than 75 min on MIP 9.

(29) Sellergren, B.; Andersson, L. I. *Methods Enzymol.* **2000**, *22*, 92–106.

Table 4. IC_{50} Values for Bupivacaine (BV), Mepivacaine (MV), and Ropivacaine (RV) on the BV MIPs and the Corresponding Relative IC_{50} Values[a]

| | IC_{50} (μM) | | | IC_{50}/IC_{50} (BV) | | |
MIP	BV	RV	MV	BV	RV	MV
11	1.6	44	388	1	27	242
9	41	1429	1777	1	35	43
14	65	140	7427	1	2.2	113

[a] Samples of polymers 11, 9, and 14 (1, 4, and 6 mg, respectively) were incubated with solutions (1 mL) each containing 50 mM citrate buffer, pH 5, 5% ethanol, 0.05% Tween 20, approximately 1.2 ng of radiolabeled Bupivacaine, and different concentrations between 1 and 330 000 nM of Bupivacaine (BV), Mepivacaine (MV), or Ropivacaine (RV). After incubation for 16 h, the supernatant was analyzed by scintillation counting. The IC_{50} values correspond to the concentration of the competing ligand (BV, RV, or MV) displacing 50% of bound radiolabeled BV.

Thus, the NIPs of polymers 9 and 14 exhibit low or nonexistent affinity for the solute, whereas the reference NIP 11 shows a steep increase in bound BV as a function of polymer concentration. The MIPs, on the other hand, all bind the solute under these conditions, although the isotherms exhibit different shapes. This is reflected in the polymer concentration (PC_{50}) required to adsorb 50% of radiolabeled BV. Whereas only 1.3 mg of MIP 11 is needed to adsorb 50% of the solute, the corresponding values for MIP 9 and MIP 14 are 4.3 and 7.2 mg, respectively. Thus, the supression of the nonspecific binding seems to have compromised the average affinity for the template. One possible explanation for this can be found by studying the structure and porosity of the materials. As expected from their lower cross-linking level, the water-compatible materials exhibit a lower surface area and pore volume compared to the

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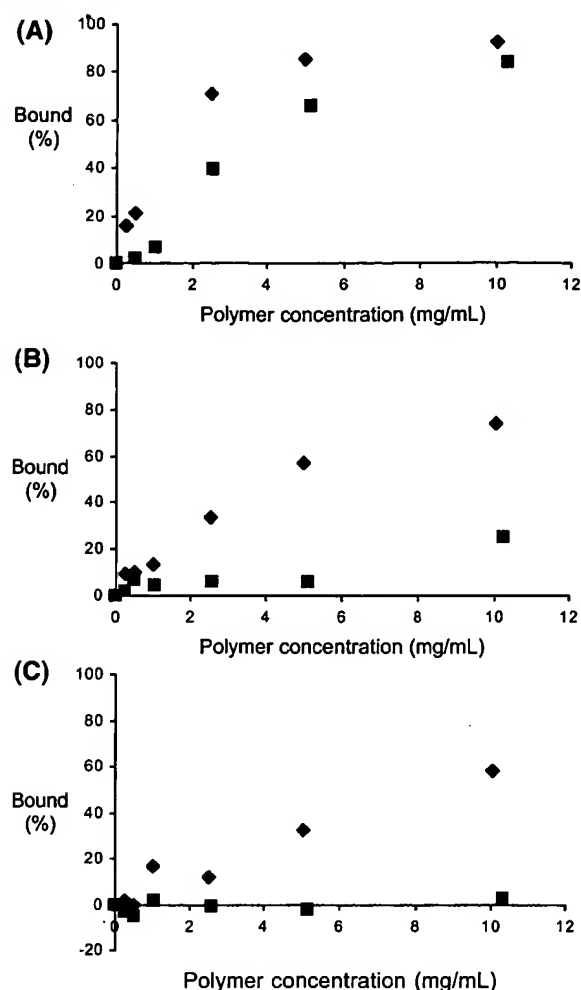
Figure 5. Fraction of bound BV in % to imprinted (diamonds) and nonimprinted (squares) polymers as a function of the amount of added polymers 11 (A), 9 (B) and 14 (C) to a 1 mL solution of BV (aproximately 1.2 ng, 30 000–50 000 dpm) in 50 mM citrate buffer (pH 5) containing ethanol (5%) and detergent (Tween 20, 0.05%).
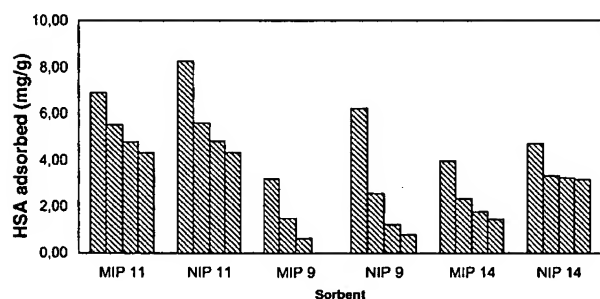
Figure 6. Amount of human serum albumin (HSA) adsorbed per gram of dry polymer sorbent in sodium phosphate buffer (25mM) at pH 7.4 after four consecutive injections (100 μL) of a standard HSA solution (50 mg/mL). The cumulative protein adsorptions were the following: MIP, 11/21 mg/g; NIP, 11/23 mg/g; MIP, 9/5 mg/g; NIP, 9/11 mg/g; MIP, 14/10 mg/g; NIP, 14/14 mg/g. The conditions were otherwise as described in the Experimental Section.

conventional polymers. This by itself can contribute to the more shallow slope of their adsorption isotherms. More alarming are the differencies in surface areas and pore volumes between the MIPs and NIPs of the watercompatible materials. The NIPs exhibit ca. 3 times lower values than corresponding MIPs whereas, for the reference materials 11, the values are similar. However, the stronger swelling of the NIPs and the similar
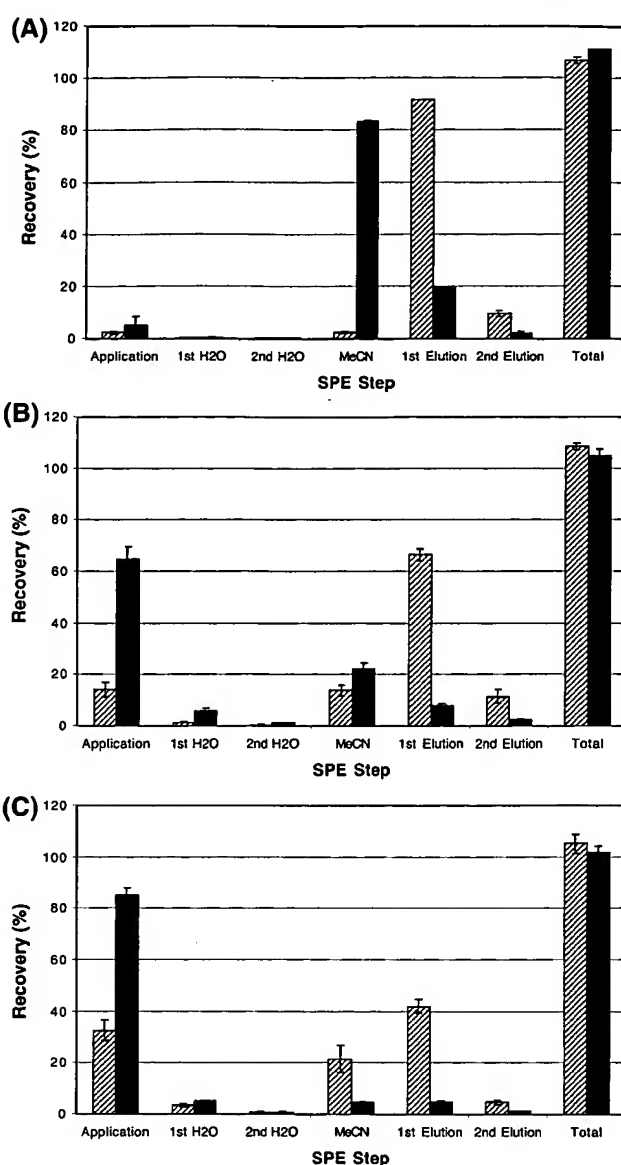
Figure 7. Recoveries of BV in each fraction obtained after solid-phase extraction of BV spiked blood plasma (1000 nM) samples of MIPs (striped bars) and NIPs (solid bars) using polymers 11 (A), 9 (B), and 14 (C) as sorbents. The sorbents (25 mg each) were packed in polypropylene SPE cartridges and subjected to the conditioning and extraction protocol described in the Experimental Section. After application of the plasma sample (1 mL), the columns were washed with 2 × 1 mL water and one time with 0.5 mL of acetonitrile followed by two elution steps with 1 mL of 92% acetonitrile, 6% water, and 2% TEA.

elution times measured for the void markers indicate that the polymers are less different regarding their swollen state morphology.[30] This highlights nevertheless the problems associated with the choice of appropriate control polymers to estimate imprinting effects. In this case, supporting evidence for the presence of templated sites is needed from selectivity assessments. We therefore performed competitive binding experiments by challenging the MIP and NIP with structurally similar compounds (Table 4).

(30) The swelling measured in acetonitrile was for MIP 9/1.19 (mL/mL) and for NIP 9/1.25 (mL/mL). The elution volume of the void marker acetonitrile after subtraction of the extracolumn volume was 13% larger for MIP 14 than for NIP 14 and 18% larger for MIP 9 than for NIP 9.

In this experiment a fixed amount of polymer is added to a dilute solution of [³H]-BV resulting in uptake of ca. 50% of the radiolabeled BV. Incremental amounts of competing ligands (BV, RV, MV) are subsequently added. The IC_{50} values correspond to the concentration of the competitive ligands required to displace 50% of bound radiolabeled BV. The lower affinity of the water-compatible polymers is further confirmed by the more than 20 times higher IC_{50} values for unlabeled BV on these polymers compared to the reference material. Nevertheless, considering the much higher IC_{50} values of the two structural analogues RV and MV on both MIP 9 and MIP 14 and the corresponding relative IC_{50} values, they exhibit selectivities on a par with the reference material MIP 11.

To prove the usefulness of the hydrophilized materials, they were tested as sorbents for direct solid-phase extraction of BV from blood plasma samples and compared with the reference materials MIP/NIP 11. Prior to the test, nonspecific adsorption of plasma proteins on the different sorbents was estimated by injecting 100 μL of a standard human serum albumin (HSA) solution (50 mg/mL) on the columns used in Figure 4. According to the results in Figure 6, polymers 11 show the lowest recovery of protein followed by polymers 14 and 9. This agrees with the expected order of decreasing hydrophobicity where polymers 11 should possess the most hydrophobic character implied by the results in Figure 5. This also implies that the surfaces of polymers 14 and 9 are likely to be less susceptible to fouling by plasma proteins.

Next, plasma samples spiked with a known amount of labeled BV (1000 nM) was applied on the different sorbents packed in solid phase extraction (SPE) cartridges. After a simple wash protocol the analyte was eluted (Figure 7). In Figure 7 the recoveries in each step expressed as an average of at least 3 extractions using two replicate columns are given. A pronounced difference in the recovery profiles can be seen. Whereas the reference MIP and NIP number 11 adsorb BV nonspecifically and almost quantitatively in the application step, polymers 9

and 14 exhibit a pronounced difference between the MIP and NIP in the same step. This difference is most pronounced for polymers 9 with more than 60% of the applied BV breaking through on the NIP whereas only ca. 15% broke through on the MIP. In agreement with the results shown in Figure 5, binding of BV to polymers 9 seems also to be overall stronger than to polymers 14.

Conclusions

High-throughput synthesis and evaluation of polymer libraries in a 96-well plate format allows rapid optimization and fine-tuning of the molecular recognition properties of molecularly imprinted polymers. This tool was successfully used to find conditions for MIP synthesis leading to reduced nonspecific binding in fully aqueous environments. Thus, MIPs selective for the local anaesthetic bupivacaine in pure aqueous buffers could be prepared. Despite the lower binding affinity of these MIPs, they exhibit high selectivity and apparently low nonspecific binding in water. This proved useful for direct and selective extraction of Bupivacaine from blood plasma samples and should prove useful as well for other biological matrixes. Furthermore, applications of such MIPs as receptor layers in chemical sensors aimed at direct determinations of analytes in aqueous samples should be feasible.

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